JOURNAL OF DAIRY SCIENCE

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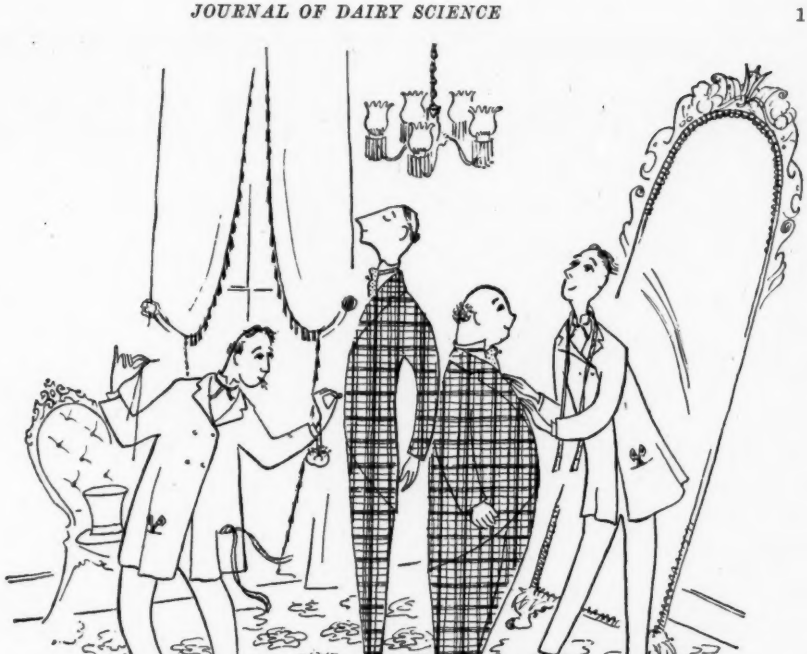

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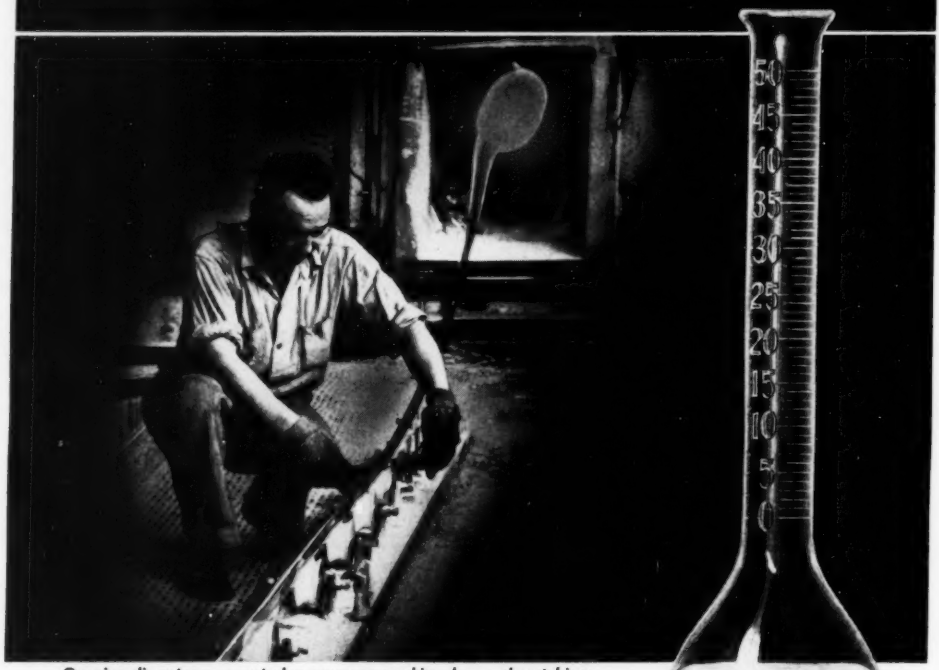

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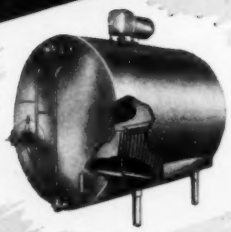

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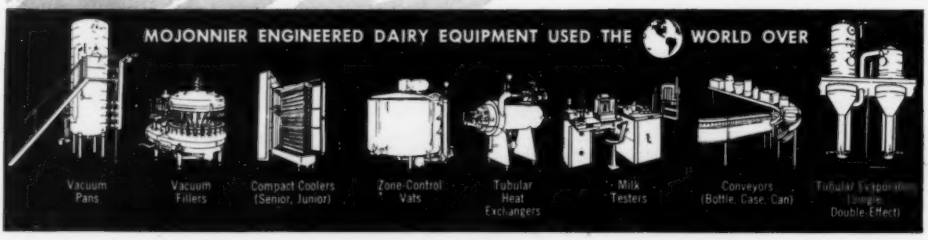

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A STUDY OF THE CHANGES OCCURRING IN THE FAT CONSTANTS OF MILK FAT OF CHEDDAR CHEESE DURING THE RIPENING PROCESS

J. J. SHEURING[1] AND S. L. TUCKEY

Department of Dairy Husbandry, University of Illinois, Urbana

The determination of the chemical changes occurring in the various constituents of Cheddar cheese and their effect on flavor development during ripening have been the object of numerous investigations. The effects on the flavor of the changes in the various nitrogenous fractions, the rate and amount of acid development, production of volatile acids, and the microflora of the cheese have received considerable attention. Less attention has been directed toward determining changes occurring in the cheese fat than in the protein fraction.

A survey of the literature did not reveal any studies pertaining to the changes occurring in the fat constants of cheese fat during the ripening process comparable with studies made on butterfat. Therefore, the purpose of the investigation reported herein was to determine the relationship between changes in the fat constants of cheese fat and flavor development of the cheese. The problem was divided into three parts to determine the effect of the following factors on changes in the fat constant values and flavor development: (a) pasteurization of the milk, (b) homogenization of the milk, and (c) the addition of organic acids to the milk and cheese curd.

REVIEW OF THE LITERATURE

The literature on cheese ripening is so extensive that only those papers pertaining to chemical changes in cheese fat will be reported. As early as 1891 Van Slyke (11) recognized that the fat of cheese changed in flavor during ripening. It was his opinion that the change in the fat was primarily hydrolytic, releasing free fatty acids. These later combined with ammonia, liberated from protein hydrolysis, to form ammonium salts of the acids. Suzuki *et al.* (10) in 1910 showed that the non-nitrogenous products

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[1] This paper represents a portion of the thesis presented by the senior author in partial fulfillment of the requirements for the M.S. degree at the University of Illinois. The research was completed before the authors entered the armed forces in 1942.

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formed during the ripening of Cheddar cheese contributed appreciably to its flavor. These investigators were the first to partition, by the Duclaux technique, the volatile acids obtained from steam distillation of Cheddar cheese. Furthermore, their results showed that: (a) Acetic and propionic acids reached a maximum at 3 months and then decreased, whereas butyric and caproic acids continually increased throughout the experimental period of 10 months. (b) The principal source of acetic and propionic acids probably was lactates, with protein and glycerine contributing only small amounts. (c) The principal sources of butyric and caproic acids were the fat and the protein. Suzuki *et al.* (10) produced a distillate having the aroma of aged cheese and containing alcohols and esters. The alcohol was primarily ethyl with esters of acetic acid and ethyl alcohol. Skim milk cheese distillate contained the esters of butyric and caproic acids and ethyl alcohol.

Lane and Hammer (6) showed that as cheese ripened the acidity of the fat increased. Furthermore, the acid values of the fat from raw milk cheese normally were higher than those of fat from cheese made from pasteurized milk. Lane and Hammer (7) later published results demonstrating that cheese made from milk standardized with raw homogenized cream and raw or pasteurized skim milk commonly developed a rancid flavor which gradually disappeared during ripening. The resulting flavor of the ripened cheese was typical. The fat acidity of this cheese was higher and its flavor more pronounced than that of cheese made from pasteurized, homogenized cream and pasteurized skim milk. However, when the rancidity was induced by pancreatin, this flavor did not disappear during ripening and the resulting product was undesirable. The addition of desiccated mammary tissue or extracts of them to pasteurized milk commonly had a desirable effect on the flavor of the cheese. Although slight rancidity occurred during the early period of ripening, it finally disappeared, producing a desirable Cheddar cheese flavor. The fat acidity was higher than that of the control, suggesting some relationship between fat acidity and development of desirable flavor. However, the authors state that fat acidity is not always a true index of a typical and desirable flavor in cheese for ". . . perhaps changes other than simple hydrolysis are necessary in the development of the typical flavor."

Babel and Hammer (2) in 1945 continued the study of fat hydrolysis in cheese and showed that, without the addition of lipase, the acidity of the fat of pasteurized milk cheese increased during the ripening period. With the addition of rennet paste, the fat acidity increased and a rancid flavor developed which frequently disappeared during ripening, leaving a desirable cheese. However, the rancidity induced by the lipase of mulberry juice when added to pasteurized milk in sufficient quantities to promote hydrolysis of the fat produced unclean flavors in the cheese.

In an attempt to standardize and determine the validity of the results of certain chemical procedures which have been used to secure the volatile acids of cheese, Hiscox and Harrison (4) made a critical study of various techniques. Their results showed that direct steam distillation of the cheese did not give a true figure for the volatile acids present in cheese, since cheese fat retarded distillation and cheese protein produced a definite retention of some of the acids. A more accurate figure could be obtained by distilling to twice the original volume than by distilling to five times the original volume. Only butyric acid could be recovered completely, regardless of the amount of distillate.

Hiscox *et al.* (5) then recommended a procedure based on water extraction of the fatty acids from cheese and ether extraction of the fat and fat-soluble fatty acids, the latter being removed by washing the ether with dilute alkali. The acids in the extractions were freed by acidifying and steam distilling.

Marquardt and Dahlberg (8), following the work of Hiscox and Harrison (4), standardized the size of cheese sample distilled and the volume of water used for distillation. Since the completion of the experimental work reported in the present paper, Smiley *et al.* (9) have simplified the procedure of Hiscox *et al.* (5). Their method employs two distillations of acid cheese solution—one associated with the fat phase and the other with the residual cheese material. The total volatile acidity is the sum of the titrations for the two distillations plus the alcohol used to rinse the condenser. Results are expressed as ml. of N/10 acid per 100 g. of cheese. The authors showed good agreement in comparing results obtained by the simplified extraction and the method of Hiscox *et al.* (5).

EXPERIMENTAL PROCEDURES

Cheese Manufacturing Methods. Fourteen samples of cheese were made and analyzed. The milk used for all the cheese was of high quality, being obtained from the University dairy herd. The different lots of milk varied in weight from 250 to 3,000 lb. The milk was clarified, tested for fat (3.8 per cent average), pasteurized in a spray vat at 143° F. for 30 minutes, and cooled to the setting temperature for cheesemaking. The manufacturing methods were such as to make a low-acid type of cheese containing 35–36 per cent moisture. However, when organic acids were added to the milk or to cheese curd, undesirable acid defects developed. The cheese was ripened at 50° F. Samples were taken for analysis at 30-day intervals, at which time the cheese also was judged organoleptically.

Chemical Methods of Analysis. The samples of fat used for chemical analysis were obtained by petroleum ether extraction of the cheese in a Soxhlet extraction apparatus. Sufficient cheese was extracted over a 4- to 6-hour period to furnish enough fat to make the various determinations. The following determinations were made on the cheese fat:

a. The acid number was determined by the method discussed by Breazeale and Bird (3) and listed as modification A of the alcoholic potassium hydroxide titration method. The acid number is expressed as the number of ml. of N/10 alkali per 10 g. of fat.

b. Reichert-Meissl number, Polenske number, saponification number, iodine number (Part III), and percentages of soluble acids (as butyric), and insoluble acids were determined by the methods of the Association of Official Agricultural Chemists (1).

c. Total volatile acidity of cheese was determined by the method of Hiscox and Harrison (4) as modified by Marquardt and Dahlberg (8). It was known that lower values would be obtained than if the refined method of Hiscox *et al.* (5) had been used. The value is expressed as the number ml. of N/10 NaOH per 200 g. of cheese.

RESULTS AND DISCUSSION

To simplify the presentation of data and results, the problem will be discussed in three parts.

Part I. The Effect of Pasteurization of the Milk upon the Fat Constant Values of Cheese Fat

To determine the effect of pasteurization of the milk on the rate of change in the values of the fat constants, four lots of cheese were made. Lots 1 and 2 represent raw milk and pasteurized milk cheese, respectively, from the same source of milk. Lots 3 and 4 also are raw milk and pasteurized milk cheese, respectively, from the same source of milk.

The analytical data of the fat for these lots of cheese are presented in table 1. The scores for organoleptic analysis of the samples are given in table 2.

From observation of the data in table 1, on the acid number values, it is apparent that there is a consistent increase for all cheese samples throughout the aging period. The percentage increase in acid numbers for the four lots was as follows: 1, 105; 2, 100; 3, 120; and 4, 87. Other workers also have found this change to occur in cheese fat. In agreement with the results of Lane and Hammer (6), the lots of raw milk cheese had higher acid numbers than did the pasteurized milk lots. Lot 1 had an acid number 55 per cent greater than lot 2. However, the acid number of lot 3 was only 20 per cent greater than for lot 4.

The Reichert-Meissl (RM) value represents a measure of the amount of volatile and water-soluble fatty acids in the fat. These consist mainly of butyric, caproic, and caprylic acids. If the fat showed appreciable hydrolysis during the ripening period, the normal trend would be for the RM number to increase. Suzuki *et al.* (10) showed that butyric and caproic acids increased throughout the ripening period in their raw milk cheese.

TABLE 1

Fat constants of cheese made from raw and pasteurized milk

Time (days)	Acid nos.				Reichert-Meissl nos.				Polenske nos.			
	Lot 1[a]	Lot 2[b]	Lot 3[a]	Lot 4[b]	Lot 1	Lot 2	Lot 3	Lot 4	Lot 1	Lot 2	Lot 3	Lot 4
1	1.47	0.97	1.12	1.13	28.80	28.70	29.41	29.92	2.67	2.83	2.70	2.24
30	1.78	1.09	1.40	1.16	.....	.....	.....	.....	.....	.....	.....	.....
60	1.43	1.19	1.52	1.28	29.35	28.65	29.65	28.58	3.47	3.42	2.46	2.00
90	1.24	1.30	1.81	1.48	.....	.....	.....	.....	.....	.....	.....	.....
120	1.55	1.33	1.89	1.56	28.80	27.95	28.95	28.50	2.45	2.40	2.47	2.00
150	1.80	1.39	2.12	1.79	.....	.....	.....	.....	.....	.....	.....	.....
180	2.10	1.43	2.16	1.85	29.97	28.80	27.50	28.25	2.35	2.76	1.82	1.64
210	2.26	1.42	2.32	1.95	.....	.....	.....	.....	.....	.....	.....	.....
240	2.86	1.49	2.42	2.07	29.93	28.75	27.40	27.15	2.20	2.25	1.77	1.55
270	3.02	1.94	2.46	2.12	.....	.....	.....	.....	.....	.....	.....	.....

Time (days)	Percentage of soluble acids				Percentage of insoluble acids				Saponification nos.			
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 1	Lot 2	Lot 3	Lot 4	Lot 1	Lot 2	Lot 3	Lot 4
1	5.03	4.80	3.82	3.85	81.30	82.00	82.20	82.50	229.90	230.00	228.00	228.00
30	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
60	5.89	5.50	5.78	4.70	79.80	85.00	80.70	83.00	228.10	228.30	226.70	225.80
90	.....	.....	.....	.....	.....	.....	.....	.....	225.70	224.00	223.80	225.00
120	4.15	4.15	4.30	4.05	79.00	82.05	82.20	84.30	.....	.....	.....	.....
150	.....	.....	.....	.....	.....	.....	.....	.....	225.00	226.60	225.90	224.50
180	4.20	4.25	4.54	4.15	77.70	83.10	83.60	82.80	224.15	226.90	228.00	221.40
210	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
240	4.10	4.10	4.40	4.25	76.60	78.80	80.70	81.00	223.30	223.70	229.00	221.40
270	.....	.....	.....	.....	.....	.....	.....	.....	220.00	221.10	229.00	221.00

[a] Raw milk cheese.
[b] Pasteurized milk cheese.

Lot 1 also demonstrated this by the increase in RM number. Lot 2 showed little change in Reichert-Meissl value, indicating only slight hydrolysis. The flavor score at 270 days was 0.5 point in favor of lot 1, the raw milk cheese which showed a slight amount of fat hydrolysis. The trend of the RM values for lots 3 and 4 was downward, instead of upward as might be expected.

The Polenske number is a measure of the volatile fatty acids, capric, lauric, and myristic, which are insoluble in water but soluble in neutral 95 per cent alcohol. The four lots of experimental cheese all showed a significantly lower Polenske value at the conclusion of the experiment, indicating a decrease in the insoluble volatile fatty acids. However, lots 1 and 2

TABLE 2

Flavor and body scores and criticisms of cheese made from raw and pasteurized milk

Time (days)	Lot 1[a]		Lot 2[b]		Lot 3[a]		Lot 4[b]	
	Score	Criticisms	Score	Criticisms	Score	Criticisms	Score	Criticisms
Flavor scores and criticisms								
30	40.0	.....	40.0	.....	40.0	.....	40.0	.....
180	40.0	.....	40.0	.....	38.5	Sl. bitter	40.0	.....
210	39.5	.....	39.5	.....	38.5	Sl. bitter	40.0	.....
270	39.5	.....	39.0	.....	39.5	.....	38.5	Sl. acidy
Body scores and criticisms								
30	29.0	.....	28.5	Sl. curdy	29.0	Curdy	29.0	.....
180	29.0	.....	29.0	.....	28.5	Curdy	28.5	Sl. mealy
210	29.0	.....	28.5	Sl. mealy	28.5	Curdy	28.5	Sl. mealy
270	29.0	.....	28.5	Sl. mealy	29.0	.....	28.5	Sl. mealy

[a] Raw milk cheese.
[b] Pasteurized milk cheese.

demonstrated an increase in Polenske number at 60 days, which was followed by a decrease in value during the remainder of the period. This perhaps showed that the fresh cheese fat was first hydrolyzed, followed by a hydrolysis of the longer chain fatty acids.

A fat which becomes hydrolyzed should show a progressive increase in soluble acids as hydrolysis proceeds, along with a decrease of insoluble acids or Hehner value. The cheese fat from each of the four lots demonstrated an increase in soluble acids at 60 days. However, at 240 days the final values for soluble acids in lots 1 and 2 were less than at the beginning of ripening. In lots 3 and 4, even though there was a consistent decrease in quantity of soluble acids, the change did not occur as rapidly as in lots 1 and 2; consequently, the final values were somewhat greater than they were at the start of the period. At 240 days, the percentages of insoluble acids in all four lots were less than at the beginning of the period. This

gives further evidence that the longer chain fatty acids were hydrolyzed during ripening of cheese.

Since the saponification number represents the number of milligrams of potassium hydroxide required to saponify 1 g. of fat, then glycerides containing low molecular weight fatty acids will have a greater saponification number than glycerides containing fatty acids of high molecular weight. Except for lot 3, the cheese fat showed a decrease in saponification numbers during the ripening period, indicating a decrease in number of low molecular weight fatty acids in the cheese fat.

An examination of the flavor scores of the cheese in table 2 shows that all cheese samples were of high quality at the beginning of the experiment. At the end of 270 days, however, the flavor of the cheese in lots 1 and 3 was slightly more pleasing. The scores for lots 1 and 3 were 0.5 and 1 point higher than for lots 2 and 4. The fat constant values for lot 1 clearly indicated a slow hydrolysis of the butterfat. This trend was not so well defined in the other samples.

Part II. The Effect of Low Pressure Homogenization of the Milk on the Change in Values of the Fat Constants

The homogenization of raw cream used to standardize pasteurized skim milk increases the acid number of the cheese fat (7). Homogenization of pasteurized milk at high pressures produces a soft curd when the milk is coagulated by rennin or pepsin. To ascertain the effect of low pressure homogenization on the fat constant values and on the body of the cheese when made from pasteurized whole milk, three lots of cheese were made.

A vat of pasteurized milk was divided into three batches and treated as follows: (a) Batch 1 was homogenized at 300 lb. pressure at 143° F. through a 200-gallon-per-hr. Multi-Flo Creamery Package homogenizer, cooled, and then made into the cheese constituting lot 5. (b) Batch 2 was treated in a similar manner except that the pressure was increased to 800 lb. Cheese lot 6 was made from this milk. (c) Batch 3 was the control without homogenization and was made into the cheese of lot 7.

All the analytical data of the fat analyses are given in table 3. The results of the organoleptic judging of body and texture are given in table 4. Observation of the data presented in table 3 shows that the acid number of the fat in all lots increased throughout the ripening period. The increase was as follows: Lot 5, 50 per cent; lot 6, 44 per cent; lot 7, 63 per cent. This increase is considerably less than was noted for the samples in Part I.

The RM numbers decreased for the fat in lots 5 and 6, made from pasteurized, homogenized milk, although the RM value slightly increased for the fat in lot 7. An increase in RM number should occur if volatile, water-soluble fatty acids were liberated.

The Polenske numbers for the fat from the different lots of cheese de-

TABLE 3

Fat constants of cheese made from homogenized and unhomogenized milk

Time (days)	Acid nos.			Reichert-Meissl nos.			Polenske nos.		
	Lot 5[a]	Lot 6[b]	Lot 7[c]	Lot 5	Lot 6	Lot 7	Lot 5	Lot 6	Lot 7
1	1.76	1.66	1.55	29.50	29.30	27.63	2.02	2.26	2.00
30	1.85	1.68	1.73	.....	.....	.....	.....	.....	.....
60	1.89	1.72	1.83	28.50	29.25	28.01	1.85	2.50	1.97
90	1.96	1.82	2.10	.....	.....	.....	.....	.....	.....
120	2.34	2.05	2.32	27.70	27.30	28.10	1.91	1.91	1.82
270	2.65	2.40	2.54	27.60	27.00	28.10	1.50	1.84	1.64
Time (days)	Percentage of soluble acids			Percentage of insoluble acids			Saponification nos.		
	Lot 5	Lot 6	Lot 7	Lot 5	Lot 6	Lot 7	Lot 5	Lot 6	Lot 7
1	5.32	5.80	5.10	81.05	84.10	82.41	227.21	227.81	221.34
30	.....	.....	.....	.....	.....	.....	.....	.....	.....
60	4.40	4.73	5.15	80.60	82.30	81.23	228.26	226.29	222.68
90	.....	.....	.....	.....	.....	.....	.....	.....	.....
120	4.62	4.62	4.23	82.50	85.72	80.16	227.74	227.83	226.53
270	4.40	4.42	4.21	83.00	82.13	79.17	228.38	228.13	226.77

[a] Homogenized at 300 lb. pressure at 143° F. through a 200-gallon-per-hr. Multi-Flo Creamery Package homogenizer, cooled, and made into cheese.
[b] Treated in a manner similar to lot 5, but homogenized at 800 lb. pressure.
[c] Control, not homogenized.

creased during the ripening period, but in lot 6 there occurred an increase in Polenske value at 60 days, followed by a decrease. This condition also took place in lots 1 and 2 in Part I.

TABLE 4

Flavor and body scores and criticisms of cheese made from homogenized and unhomogenized milk

Time (days)	Lot 5[a]		Lot 6[b]		Lot 7[c]	
	Score	Criticisms	Score	Criticisms	Score	Criticisms
	Flavor scores and criticisms					
1	39.0	.....	39.0	.....	39.0	.....
60	38.0	Sl. cooked	38.0	Sl. cooked	38.5	Sl. bitter
120	38.5	Sl. acidy	40.0	.....	38.0	Bitter
270	39.0	.....	38.0	Sl. acidy Sl. bitter	38.5	Sl. bitter
	Body scores and criticisms					
1	29.0	.....	28.5	Sl. curdy	29.0	.....
60	28.5	Sl. weak	28.5	Sl. curdy	29.0	.....
120	29.0	Sl. open	29.5	.....	29.0	.....
270	28.5	Sl. mealy	28.0	Sl. pasty	28.5	Sl. weak

[a] Homogenized at 300 lb. pressure at 143° F. through a 200-gallon-per-hr. Multi-Flo Creamery Package homogenizer, cooled, and made into cheese.
[b] Treated in a manner similar to lot 5 but homogenized at 800 lb. pressure.
[c] Control, not homogenized.

The trend of the values for both the soluble and insoluble acids was downward. The percentage of insoluble acids would be expected to decrease in proportion to the amount of hydrolysis occurring in the longer chain fatty acids. Moreover, the soluble acids might be expected to increase as a result. The decrease, however, in percentage of soluble acids for lots 5 and 6 is in agreement with the trend of the RM numbers for these samples.

The saponification numbers of the fat from lots 5, 6, and 7 showed an increase during the ripening period. The reverse of this trend occurred with lots 1, 2, 3, and 4.

An examination of the flavor scores for lots 5, 6, and 7 (table 4) indicates that the flavor of lot 5 was superior to the others at the end of 270 days. Lot 6 was the least desirable, for it had a bitter flavor and a pasty body. There was little difference, however, between lots 5 and 7.

Except for the acid numbers and Polenske numbers, the other fat constant values do not present clear evidence of significant fat hydrolysis.

Part III. The Effect of the Addition of Various Organic Acids upon the Fat Constant Values and Volatile Acidity and Flavor of Cheese

To determine the effect of the addition of various organic acids upon the fat constant values, volatile acidity of the cheese, and flavor of the cheese, seven lots were made. Two-hundred fifty pounds of pasteurized milk were used for each lot and the substances added were as follows: Lot 8, control; lot 9, 20 ml. of propionic acid added to the milk at time of setting; lot 10, 15 ml. of glacial acetic acid added to the curd at time of salting; lot 11, 15 ml. of glacial acetic acid added to the milk at time of setting; lot 12, 1 l. of 2 per cent citric acid added to the milk at time of setting; lot 13, 1.5 pints of starter distillate added to the milk at time of setting and 0.5 pint of starter distillate to the curd at salting; lot 14, 10 ml. of propionic acid, 10 g. of citric acid, and 14 ml. of glacial acetic acid dissolved in 1 l. of water added to the milk at setting. The different lots of cheese were not made from the same milk. However, during the period of time the cheese was made, no significant changes occurred in source of milk.

The analytical data for the fat constants of the seven lots are presented in table 5. The judging scores for the various lots are listed in table 6. Observation of the data on acid numbers of the cheese fat, as given in table 5, shows the same trend as those samples in Parts I and II. The percentage increase in acid values for the samples was as follows: Lot 8, 60; lot 9, 67; lot 10, 218; lot 11, 73; lot 12, 71; lot 13, 42; and lot 14, 76. A marked effect was produced in lot 10 when acetic acid was added to the curd at time of salting. The hydrolysis of the fat was activated to a pronounced degree, for by the end of the experiment an increase of over 300 per cent in acid value had occurred. When acetic acid was added to the milk in lot 11, the acid number of the fat was high at the beginning, so the percentage increase

TABLE 5

Fat constants of Cheddar cheese containing added organic acids and starter distillate

Time (days)	Acid nos.							Reichert-Meissl nos.							Polenske nos.						
	Lot 8[a]	Lot 9[b]	Lot 10[c]	Lot 11[d]	Lot 12[e]	Lot 13[f]	Lot 14[g]	Lot 8	Lot 9	Lot 10	Lot 11	Lot 12	Lot 13	Lot .14	Lot 8	Lot 9	Lot 10	Lot 11	Lot 12	Lot 13	Lot 14
1	1.74	0.94	0.90	1.72	0.83	1.73	0.88	27.55	27.36	29.05	27.70	26.30	27.40	27.93	2.16	1.78	2.13	2.06	1.75	2.09	1.84
45	1.81	1.05	1.22	1.98	1.25	1.83	1.13	27.32	27.20	27.90	27.22	26.22	27.80	28.02	1.96	1.82	2.32	2.19	1.85	1.79	1.91
90	1.98	1.17	1.40	1.90	1.12	1.82	1.26	27.50	26.27	27.35	28.30	26.75	27.95	26.65	1.96	1.70	2.13	1.75	1.79	1.55	1.93
135	2.18	1.19	1.43	2.16	1.13	2.09	1.20	28.68	27.30	27.30	28.65	27.90	29.05	25.82	1.85	1.72	2.10	1.68	1.84	1.78	1.72
225	2.44	1.26	1.58	2.40	1.35	2.18	1.41	29.85	27.70	29.22	28.65	27.70	29.50	27.40	1.74	1.90	1.55	1.54	1.74	2.01	1.92
270	2.79	1.57	2.87	2.98	1.42	2.47	1.55	29.92	28.00	29.35	28.93	27.46	29.62	28.75	1.72	1.76	1.43	1.63	1.64	2.38	1.97

Time (days)	Percentage of soluble acids							Percentage of insoluble acids						
	Lot 8	Lot 9	Lot 10	Lot 11	Lot 12	Lot 13	Lot 14	Lot 8	Lot 9	Lot 10	Lot 11	Lot 12	Lot 13	Lot 14
1	2.10	4.30	2.84	3.37	2.90	3.60	3.40	85.52	84.31	82.56	83.73	84.71	82.47	87.72
45	2.67	4.40	2.98	3.68	2.88	3.97	3.58	84.31	86.62	82.03	85.52	84.02	84.40	86.12
90	3.18	4.56	3.16	3.97	3.16	4.05	3.62	84.18	85.04	82.14	85.43	85.68	84.62	84.92
135	3.05	4.40	4.47	4.90	4.04	4.10	3.78	84.14	84.26	82.19	84.89	82.61	84.72	84.18
225	4.82	4.76	5.19	5.82	4.03	4.82	3.91	84.02	83.19	82.33	83.92	80.98	84.13	85.13
270	5.23	4.68	6.18	6.28	4.15	4.71	4.02	83.65	80.63	82.41	83.64	78.52	83.78	86.16

Time (days)	Saponification nos.							Iodine nos.						
	Lot 8	Lot 9	Lot 10	Lot 11	Lot 12	Lot 13	Lot 14	Lot 8	Lot 9	Lot 10	Lot 11	Lot 12	Lot 13	Lot 14
1	222.73	222.16	221.42	222.18	222.24	222.18	222.72	29.62	32.71	33.42	30.82	33.99	30.04	35.52
45	222.98	221.13	223.16	223.52	220.16	221.15	220.32	.....	.....	.....	.....	.....	.....	.....
90	221.53	221.10	225.18	225.19	220.98	221.74	220.18	.....	.....	.....	.....	.....	.....	.....
135	222.64	220.52	228.16	226.89	223.64	222.34	223.13	28.81	32.18	34.10	26.83	33.86	29.55	35.74
225	222.98	223.18	228.03	228.53	225.18	221.19	226.43	.....	.....	.....	.....	.....	.....	.....
270	223.13	224.19	223.45	228.55	226.17	221.12	228.87	27.74	32.03	31.64	25.74	31.64	28.81	32.05

Time (days)	Volatile acid nos.						
	Lot 8	Lot 9	Lot 10	Lot 11	Lot 12	Lot 13	Lot 14
1	12.04	8.05	19.42	10.70	7.92	12.34	10.42
45	16.50	10.20	19.88	17.25	10.34	16.50	10.33
90	14.50	11.80	19.61	20.90	12.55	18.12	10.43
135	12.20	11.60	17.02	16.83	11.56	17.64	10.22
225	9.80	8.60	14.81	12.89	9.02	16.88	9.68
270	8.68	6.90	13.24	11.40	9.24	14.96	8.44

[a] Control.
[b] 20 ml. of propionic acid added to the milk at time of setting.
[c] 15 ml. of glacial acetic acid added to the curd at time of salting.
[d] 15 ml. of glacial acetic acid added to the milk at time of setting.
[e] 1 l. of 2% citric acid solution added to the milk before adding rennet.
[f] 1.5 pints of starter distillate added at the time of adding rennet and 0.5 pint of starter distillate added to the curd at time of salting.
[g] 10 ml. of propionic acid, 10 g. of citric acid, and 14 ml. of glacial acetic acid in 1 l. of water added at the time of setting.

during ripening was less marked. Lot 13, containing the starter distillate, showed a high acid number at the beginning of the period. The smallest increase during ripening occurred in this sample. The control (lot 8) also showed a high initial acid number.

In general, the water-soluble, volatile acids of the fat for all samples increased during the ripening period. This condition also is confirmed by the increase in RM values as listed in table 5. The increase in RM values was more consistent for the samples in Part III than for samples in Parts I and II. However, the RM values for lot 10 decreased during a 3-month period and then increased again during the last 135 days of ripening.

The Polenske numbers tended to decrease for the majority of samples during ripening. This trend would indicate a decrease in quantity of insoluble, volatile acids. Since the volatile, soluble acids increased at the same time, apparently the glycerides containing long-chain fatty acids were hydrolyzed.

The percentages of soluble acids in all lots of cheese increased throughout the ripening period, presenting further evidence of fat hydrolysis and liberation of low molecular weight fatty acids. The percentages of insoluble acids showed a tendency to decrease, although it is not as well defined as the trend of the soluble acids. The saponification numbers for the different lots showed a tendency to increase. The data on the changes of the iodine number of the cheese fat are inconclusive.

The total volatile acidity of the cheese reached a maximum value, except for lots 8 and 13, at 90 days. Suzuki *et al.* (10) demonstrated that acetic acid and propionic acid reached a maximum value at 90 days and then decreased. The experimental lots of cheese, 10, 11, 13, had the greatest volatile acidity. Lots 10 and 11 contained acetic acid. The drop in volatile acidity is especially apparent at the time the samples were analyzed at 225 days.

At the end of the experiment, samples 12 and 13, to which had been added citric acid and starter distillate, respectively, had the highest flavor score (table 6). The samples containing the added propionic acid and acetic acid showed abnormal and undesirable bitter flavors from the beginning of the experiment. Lot 10, in particular, quickly developed into an undergrade cheese. Due perhaps to the abnormally high amounts of organic acids added to the milk and cheese, their effects on flavor were so pronounced that desirable effects that might have been produced with smaller quantities quickly were masked by the flavor defects which developed.

SUMMARY AND CONCLUSIONS

A study has been made of the changes occurring in the fat-constant values of cheese fat during the ripening of Cheddar cheese. Fourteen lots were made and analyzed at regular intervals during an aging period of 9 months. The study was divided into three parts to determine the effect of

TABLE 6

Flavor and body scores and criticisms of Cheddar cheese containing added organic acids and starter distillate

Time (days)	Lot 8[a]		Lot 9		Lot 10		Lot 11		Lot 12		Lot 13		Lot 14	
	Score	Criticism	Score	Criticism	Score	Criticism	Score	Criticism	Score	Criticism	Score	Criticism	Score	Criticism
Flavor scores and criticisms														
1	39.5	.....	37.0	Propionic	37.0	Bitter	39.0	.....	39.0	.....	39.5	.....	37.5	Fruity
45	39.0	.....	37.5	Pro. fruity	36.0	Bitter	39.0	.....	38.5	Sl. cooked	39.0	.....	37.5	Acidy
90	39.0	.....	37.5	Propionic	36.0	Bitter	38.0	Bitter	38.5	Sl. cooked	39.0	.....	37.0	Fruity
135	39.0	.....	37.0	Pro. bitter	36.0	Bitter	38.5	Bitter	38.5	Sl. bitter	38.0	Acidy	37.5	Bitter
225	38.0	Sl. bitter	37.0	Pro. fruity	35.0	Bitter	37.5	Bitter	38.5	Sl. bitter	38.5	Sl. acidy	37.5	Bitter
270	37.5	Bitter	37.5	Pro. fruity	35.0	Bitter	37.0	Bitter	38.5	Sl. bitter	38.5	Sl. acidy	37.0	Bitter
Body scores and criticisms														
1	29.0	.....	28.5	Sl. curdy	27.0	Soft	29.0	.....	28.5	Sl. curdy	29.0	.....	28.5	Weak
45	29.0	.....	28.0	Weak, curdy	27.0	Soft	29.0	.....	28.0	Curdy	29.0	.....	27.0	Weak
90	29.0	.....	28.5	Sl. curdy	28.0	Pasty	29.0	.....	29.0	.....	29.0	.....	27.0	Weak
135	29.0	.....	29.0	.....	27.5	Mealy	28.5	Weak	29.0	.....	28.5	.....	28.5	Sl. crumbly
225	29.0	.....	28.5	.....	27.5	Mealy	28.5	Weak	28.0	Weak	28.5	Sl. open	28.5	Sl. crumbly
270	28.5	Sl. mealy	28.5	.....	27.0	Pasty	28.5	Weak	28.5	Weak	28.5	Sl. open	28.5	Weak

[a] See table 5 for footnotes.

pasteurization of the milk, homogenization at low pressures of pasteurized milk, and addition of organic acids to milk.

The results obtained in Part I show that pasteurization of the milk used for Cheddar cheese reduced the hydrolysis of the fat in the cheese. Hydrolysis of the cheese fat, as indicated by an increase in acid number, RM, and a decrease in Polenske number, was greatest in lot 1 made from raw milk. The flavor of the cheese in lot 1 was slightly more pleasing at 9 months than that of the cheese made from pasteurized milk.

The results obtained in Part II show that homogenization of pasteurized milk at low pressures produced no significant changes in the fat constants of the cheese fat. Homogenization of the milk at 800 lb. pressure produced a weak pasty body in the cheese after aging for 9 months. A pressure of 300 lb. exerted no detrimental effect on the body.

The results obtained in Part III show that the addition of organic acids accelerated the hydrolysis of cheese fat. Acetic acid added to the cheese curd in lot 10 caused more than 300 per cent increase in acid number during aging. Other samples, along with the control lot, showed a 60 to 70 per cent increase. The quality of lot 10 was unusually low, the flavor extremely bitter, and the body weak and pasty.

The RM values for the samples were uniformly higher at the conclusion of the study than at the beginning, indicating an increase in water-soluble, volatile fatty acids during aging. The saponification numbers also tended to increase. The Polenske values, on the other hand, decreased during the same period, showing that water-insoluble, volatile acids were being reduced. The percentages of soluble acids increased and the percentages of insoluble acids tended to decrease, giving further evidence of hydrolysis of the cheese fat.

The maximum values of the total volatile acidity of the cheese samples, except lots 8 and 10, occurred at 90 days and then decreased. These two lots showed greatest total volatile acidity at 45 days.

The data on the iodine numbers are inconclusive. The variation in values from the same sample was so wide the results were not of use.

The addition of acetic and propionic acids in the quantity used produced undesirable defects in the flavor of the cheese. The lots of cheese containing citric acid and starter distillate had the highest flavor score at the conclusion of the ripening period.

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FERTILITY LEVEL IN ARTIFICIAL BREEDING ASSOCIATED WITH SEASON, HOURS OF DAYLIGHT, AND THE AGE OF CATTLE

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Under semi-feral conditions where bulls remain with the cow herd the year around, mating takes place during late spring and summer months. While animals might breed at other times of the year, there is an optimum breeding period when the reproductive powers are at a maximum.

Dairy cows under farm conditions may breed the year around, though there is a seasonal peak of calvings in the spring in most areas. In New York State dairymen complain about the difficulty experienced in getting cows with calf during winter so as to have them freshen in the fall, especially in the case of virgin heifers and old cows. Furthermore, reports have been more frequent recently of artificial breeding organizations experiencing difficulties in collecting semen of satisfactory quality from some bulls during winter.

In studies conducted by Salisbury (8) and by Mercier and Salisbury (5), data on 16 of the 40 bulls could not be used for analyses because fertility data were not available for at least 11 months during the year. The fact that of these 16 bulls 5 were young (36.6 ± 20.8 month of age) and 11 were aged bulls (94.4 ± 14.8 months of age) raised the question of whether the fertility level of bulls of extreme ages might be influenced more readily by seasons than that of bulls of medium age.

None of the publications reviewed earlier (6), nor the paper by Trimberger and Davis (11) dealing with artificial breeding, considered the possibility of differential responses of animals of varying ages to the cyclic climatic changes which occur throughout the year. The diverse results of published studies concerning the effect of season on reproduction in cattle, whether for natural or artificial breeding, do not enable one to determine if an age differential is involved. The interaction between the age of bulls and cows in determining fertility level, as reported by Tanabe and Salisbury (10), is difficult to interpret directly on physiological grounds. If an age-by-season interaction for fertility level were demonstrated, their results might be shown to be due to variations in proportion of individuals of the several age classes bred during the different seasons of the year.

Finally, if bulls of varying ages responded differently to the effect of

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climatic variations associated with the seasons of the year, the over-all fertility level in artificial breeding might be controlled more satisfactorily by use of bulls of certain ages during particular seasons of the year. The authors hoped to obtain information on which semen collection schedules could be based for bulls of various ages, in order to maintain them in fertile service longer and to obtain a maximum fertility level the year around.

The present report deals with studies on the seasonal fluctuations in fertility level of cows and of bulls of various age groups. In view of the relationship shown between hours of daylight and fertility level for Eastern Canada (6), the situation under New York conditions also was considered.

EXPERIMENTAL PROCEDURES

The data for these studies were collected from the breeding records of the New York Artificial Breeders' Cooperative, Inc., the headquarters of which are located in Ithaca, New York. The bulls were located in Ithaca, but the cows were located on 19,000 or more farms throughout the State.

Cows: The study of cows where the age was verified included 14,713 recorded services for purebred Holstein-Friesian cows bred during a 4-year period from the beginning of the association in June, 1940, until June 30, 1944. Of these services, 8,284 were services to cows bred for the first time for any single service period, 4,206 were second services or services to cows which did not conceive from the first insemination, and 2,223 were third services or services to cows which failed to conceive from a second insemination.

Registration numbers from the Holstein-Friesian Herd Books were used to determine the date of birth of each individual cow.[2] Cows arbitrarily were divided into four groups: those under 39 months, those from 39 to 63 months, those from 63 to 90 months, and those over 90 months of age. The age interval chosen was believed to correspond roughly to two consecutive conceptions in the two younger groups. The basis for this classification was the time interval found between 1,163 calvings (372 ± 46 days) of 406 cows in 12 purebred dairy herds enrolled in Dairy Herd Improvement Associations in seven New York counties. Whether or not this calving interval corresponds closely to that of purebred Holstein-Friesian cows in the State is not known, although it probably is shorter than that commonly found.

Data were sorted by months according to the age of the cows bred, regardless of the age of the bulls from which the semen was used. Monthly values were grouped into seasons, winter months being December, January, and February; spring, March, April, and May; summer, June, July, and August; and fall, September, October, and November.

The conception rate of the cows was determined indirectly by the number

[2] The authors are greatly indebted to Tsuneo Tanabe for determining the age of the cows.

of successful services or the per cent of cows not coming back to service within a 5-month period after insemination. The value obtained corresponds closely to the actual percentage of conceptions as determined by the number of calves born (3) or the pregnancy diagnosis (1) and commonly is spoken of as the per cent of 5 months' non-returns.

Bulls. The studies on bulls include 116,881 first services from 42 Holstein-Friesian and 29 Guernsey bulls used from the time the headquarters of the Cooperative were established in Ithaca, December, 1944, through Novmber, 1946. Second and third services were not considered.

The age of the bulls was determined from their birth dates and they were classified in four age groups: under 4 years of age, from 4 to 6, from 6 to 8, and over 8 years of age. The level of fertility of each male was based on the per cent of cows bred to him which were not rebred within 60 days after the first insemination, regardless of the age of these cows. The decrease in the per cent of non-returns between 2 months and 5 months is about 5 percentage units (9) and this value must be subtracted from the per cent of 2 months' non-returns to obtain a figure corresponding to the actual number of conceptions. For these studies the age of each cow was not available.

Analysis. In each study the data were grouped by months. In the case of the cows they were grouped further according to the age of the cow involved. For the bulls the data were arranged under the individual bulls and these individuals classified by age groups. For the cow data, the significance of differences between means was determined by covariance analysis using the number of cows bred as the independent variable and number of cows not returning to service as the dependent variable. This was done because in some of the age groups less than 30 cows were bred during one or another of the fall months. Cochran (2) has studied data of this type and cautions against the use of percentages where the calculated values are based on small numbers. In the data for bulls this problem was not found, for the average bull was bred to nearly 200 cows a month, so that analysis of variance of per cent non-returns was used.

RESULTS

Age and conception rate of cows. The data on conception rate of cows, including those on cows bred for the first and second time in any service period, are shown in figure 1. They indicated that the two extreme age groups fluctuated more in fertility level from season to season than did the cattle of intermediate ages. The over-all conception rate for all groups of cows was low in winter, somewhat higher in summer, and highest during spring and fall. However, in the statistical analysis of the data, the difference between the means for the four seasons was not significantly different when tested by the age × season interaction.

The age × season interaction, on the other hand, was significantly larger than was to be expected on the assumption that it was no different than the month to month variation within seasons and within the age groups. Thus, the conclusion was reached that there were marked differences between seasons in fertility level of cattle of a particular age. However, one age group performed differently from another. Whether or not a real seasonal difference in fertility level would occur in a population of cows would depend on the proportion of cows of the various ages in that population.

A study of the number of cows in each age group bred in each season showed that, in spite of a real attempt by dairymen to breed their cows for

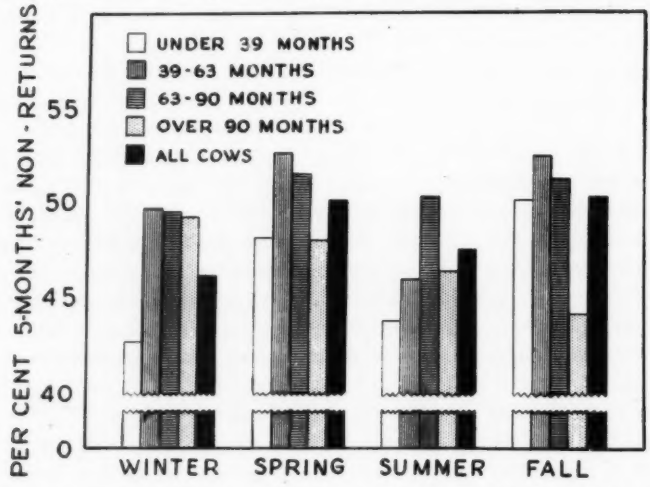

FIG. 1. Seasonal variations in fertility level of purebred Holstein-Friesian cows of different ages bred artificially in New York, June 1, 1940, to June 30, 1944.

fall freshening, as cows grew older a higher proportion of them were bred in the summer. For example, among cattle less than 39 months of age, 19.3 per cent were bred in June, July, and August. For those cows over 90 months of age, 27.9 per cent were bred in those 3 months. Thus, not only does fertility level vary from season to season in a group of cows of a particular age, but so does the proportion they constitute of those cows being bred in a particular season. Roberts (7), in England, observed an increase in the proportion of spring calvings, as a consequence of an increase in summer conceptions, as cows advanced in age. This fact and practical experience support our observations.

Fertility level of bulls. The first of these studies on seasonal variations in fertility level of bulls was made on all Holstein-Friesian and Guernsey bulls used during the 2-year period, December, 1944, through November,

1946. The data are summarized for seasons and age groups in figure 2. They represent 2 months' non-returns on 116,881 first services to cows of all ages.

Analysis of variance showed that the differences in fertility level between seasons were significant at the 5 per cent level of probability when tested against the season × age interaction, which in itself was not different from the random variability in the data.

By far the heaviest use of bulls was in the 6- to 8-year-old group, which fact biased the study in the direction of determining seasonal effects within

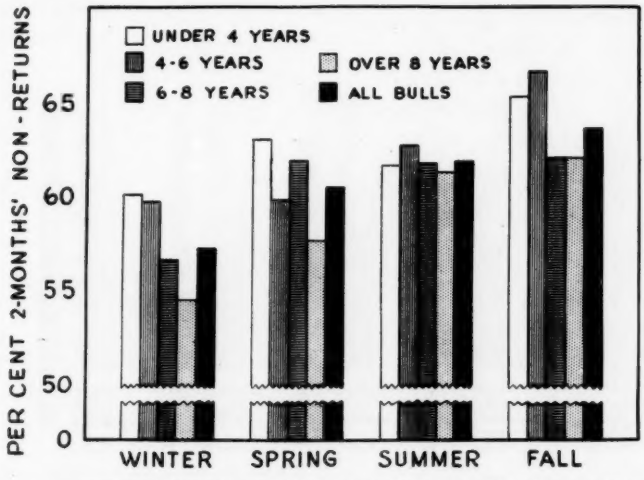

FIG. 2. Seasonal variations in fertility level of 42 Holstein-Friesian and 29 Guernsey bulls of different ages used in artificial breeding in New York at any time during the period from December, 1944, through November, 1946.

one age group. In order to obviate this difficulty, all bulls of the several age classifications which had been used consistently month after month throughout the 2-year period were selected for further study. An equal number of bulls in each age group was chosen at random from the numbers available. There were available for study three bulls in each of the four age groups, or a total of 12. A summary of the results, representing 39,833 first services to cows of all ages, is given in figure 3.

On the average, the same general trends are shown in both figures. Fertility level is the lowest in winter, gradually improving during the spring to reach a high level in the summer and fall. However, this second study, illustrated in figure 3 and orthogonal with respect to age groups, shows that each age group responded differently to season. The young bulls were relatively high in fertility in winter, gradually decreasing to a low in the hot

weather of summer. Bulls in the oldest group were lowest in fertility in winter, gradually increasing in fertility level to reach a maximum in summer several weeks after the longest day of the year. Statistical analysis of these data showed a highly significant season × age group interaction when tested by the remaining variance. However, because of the size of the variance associated with this interaction, the differences observed between seasons were not found to be statistically different when tested by it.

Finally, from the total of 71 bulls used during this time interval, it was possible to select three bulls at each age interval from each of the two

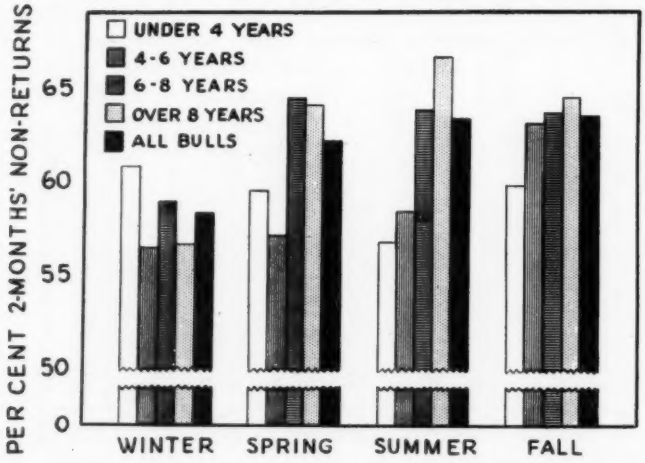

FIG. 3. Seasonal variations in fertility level of three bulls in each of four age groups used in artificial breeding in New York consistently month by month during the period from December, 1944, through November, 1946.

breeds which had been used each of the four seasons of the year, commencing in December, 1945. In some cases fertility evidence was not available each month of the year but was available for a portion of each season. This study, then, included a total of 24 bulls, 12 of each breed, and a total of 49,908 first services to cows of all ages. The data reflect an intermediate position between figure 2 and figure 3. As no significant breed by season interaction was shown, the evidence is not presented here. The fertility level of the Guernsey bulls was slightly higher than that of the Holstein-Friesian bulls, a fact which is contrary to the observations noted earlier (5).

It is of interest to note that proper temperature control of the semen during the summers of 1945 and 1946, by use of the semen carrying chest, eliminated the low fertility results during summer observed earlier and shown in figure 1.

Hours of dayight and fertility level. In view of the relationship between

the hours of daylight and the monthly level of fertility observed earlier for Canadian conditions (6), correlation coefficients between these items for groups of bulls of various ages were determined. The records used were from December, 1945, through November, 1946, and include data on five bulls in each age group used regularly each month of the year, as well as data on all bulls used throughout the year. In order to get an idea of the length of the apparent lag effect of daylight variations, correlation coefficients between fertility level for one month and the average length of daylight for the month corresponding to and for the first, second, and for the third month prior to service were determined. The correlation coefficients are presented in table 1. No significant correlation between these two variables was observed for bulls under 6 years of age. It was high for bulls of 6 to 8 and 8 to 10 years of age, low for bulls over 10 years of age, and high for all bulls. The correlation between fertility level and length of daylight, on the whole, was highest for the first and second month prior to service.

TABLE 1

Correlation coefficients between monthly fertility level of bulls of various ages and the length of daylight in Ithaca, N. Y.

Age of bulls (yr.)	Months for length of daylight							
	Corresponding		Prior to services					
			First		Second		Third	
	5 Bulls	All	5 Bulls	All	5 Bulls	All	5 Bulls	All
Under 4	0.29	0.38	0.30	0.35	0.22	0.22	0.05	0.00
4–6	0.06	0.16	0.25	0.36	0.40	0.48	0.44	0.49
6–8	0.36	0.27	0.65*	0.56*	0.75†	0.69†	0.64*	0.63*
8–10	0.47	0.64*	0.47	0.71†	0.30	0.56*	0.08	0.28
Over 10	0.45	0.66*	0.56*	0.68*	0.52	0.53	0.33	0.25
All bulls	0.48	0.56*	0.67*	0.75†	0.67*	0.75†	0.49	0.51

* Significant at the 5% level of probability.
† Significant at the 1% level of probability.

The over-all relationship between these two variables under Canadian and New York conditions agrees closely. The Canadian results involve records from three herds for 25 years on cattle of all ages bred normally. The New York results involve records collected during 1 year from 19,000 or more farms where cattle of all ages were bred artificially.

DISCUSSION

Because of the enormity of the task, it was impossible to establish the age of both the bull and the cow for each service involved in these studies. Such data would enable one to determine the possible seasonal interactions in fertility of mates of the same and of different ages. The fertility interaction

between the various ages of bulls and cows, as observed by Tanabe and Salisbury (10), might be explained on that basis.

Also, it would have been desirable to have the same uniform schedule of semen collection for all bulls involved in these studies. However, this was impractical because of the limited number of bulls available and of the seasonal demand for semen. No significant relationship between fertility and the same general frequency of semen collection was observed earlier under New York conditions (5).

Nevertheless, the results obtained in this investigation show that with respect to fertility level, males and females of various ages, regardless of the age of their mates, responded differently and consistently to the seasons of the year. The fertility level of younger and older cattle fluctuated more from season to season than that of cattle of medium age.

Winter was the poorest breeding season of the year for all age groups at this latitude. The fact that one-third of the yearly number of artificial services in New York occurs during winter when the fertility level of cattle is at its minimum creates a serious problem. This management practice decreases the over-all fertility level. One way to improve this condition would be to breed young cows during early spring and fall, cows of medium age during winter, and old cows during late spring and summer, if this practice did not upset the seasonal distribution of calvings desired. Young bulls probably should be used least during the summer, and may be used advantageously during the winter, when the fertility of older bulls is at a low level.

In an earlier publication (5) the authors obtained results dealing with the semen production and fertility level of a group of relatively young bulls which showed the poorest fertility level in the summer, in spite of the fact that the temperature of the semen was maintained properly. Those results would be expected on the basis of the results obtained here with bulls of equivalent age. However, if only the average results for all bulls in each case are compared, the fertility levels shown for the seasons are quite different. In their earlier review of the literature (5), the present authors stated that there was a lack of unanimity among investigators concerning effect of season on reproductive performance in bulls. The present results, showing an interaction of season and age of bulls, resolve the disagreement, and the earlier studies by others support the picture as described.

As discussed earlier (6), the authors believe that light influences reproductive functions in cattle by its action on the pituitary through the eye and the optic nerve. The different reproductive responses of cattle of various ages to seasons are believed to be due to a varying priority need for pituitary substances by various physiological functions of the body. This theory does not exclude the possibility that other endocrine organs subordinated to the pituitary might be involved in the differential response of cattle of various

ages to the season of the year. Work needs to be done to demonstrate the mechanism of light on fertility level of cattle of various ages, and to test the possibility of using artificial light to improve the fertility level of cattle bred during winter.

SUMMARY

In an investigation on the seasonal variations in fertility level of about 125,000 cows and 71 bulls of various ages bred artificially within the last 6 years in New York State, it was found that:

1. With respect to fertility level, cattle of various ages responded differently and consistently to seasons of the year, the younger and older cattle being influenced more readily than mature cattle.

2. Winter was the poorest breeding season of the year during this period.

3. The fertility level of all bulls kept at this Station but artificially bred to cattle in all sections of the State from December, 1945, through November, 1946, was significantly correlated with the length of daylight, there being a lag of 1 or 2 months before the effect of daylight reached its maximum. For two groups of bulls less than 6 years of age, the correlation coefficients were not statistically significant. Thus, young bulls probably are more subject to other extraneous influences on fertility than are those from 6 to 10 years of age.

It is concluded that variations in the hours of daylight influence the fertility level of dairy cattle at this latitude, and that the response to light varies with the age of the animals involved.

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RATING THE ACID-PRODUCING ABILITY OF STARTERS FOR CHEDDAR CHEESEMAKING[1]

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The purpose of adding starter to milk in the manufacture of Cheddar cheese is to induce the development of lactic acid at a dependable rate and to secure thereby a maximum measure of control of the vat. To achieve this end, a knowledge of the behavior of the starter *per se* is fundamental.

Whitehead and Cox (9) developed a method of determining the vitality of starters which consists of simulating, in miniature, the various steps in the cheese vat. The increase in the titratable acidity of whey between the fifth and sixth hours is taken as the vitality of a starter relative to another starter treated in the same way. Babel (1) used the acidity attained at intervals of 1 or 2 hours during 7 hours under specified conditions as an index of the acid-producing ability of starter cultures.

A somewhat more fundamental approach, with definite advantages, can be made to the problem of rating the acid-producing ability of starters. The lactic acid-producing property of starter organisms basically is the effect of one or more enzyme systems. It is logical to assume that under defined conditions rate of acid production should be characteristic of that starter.

Before proceeding further, a clear distinction should be made between the scientific use of the word *rate* and its somewhat broader meaning. For instance, in their review of butter starters, Hammer and Babel (7) state that ". . . the rate of a ripening culture is controlled primarily by varying the amount of inoculum . . .". Similarly, in his bulletin, Hales (6) states that ". . . the amount of inoculation affected the rate of acid development . . .". These and other authors refer to a static concept implying the over-all rate or the time required for a culture to reach a maximum acidity.

When used more precisely, the word *rate* is a dynamic concept. This usage may be illustrated by referring to a discussion of the growth phases of bacteria in any standard book on bacteriology. The rate of growth keeps changing from a stationary, through accelerating, maximum, decelerating, and, finally, stationary phases. Graphically it is indicated as the slope of the curve.

According to this second concept, the data of Monrad and Helmer, reproduced by Hales (6), show that the rate of lactic acid production measured as titratable acidity is independent of the amount of inoculum used. All the curves shown are practically identical in shape but are merely displaced

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on the time axis. Similarly, Dolby (4) showed that the rate of lactic acid development in the cheese curd, measured as pH, is independent of the amount of starter used.

In order to characterize a starter by its rate of lactic acid production, it is necessary to establish a relationship between acid development and time. It was indicated by Baker *et al.* (2) that the rate of lactic acid development, whether measured as titratable acidity corrected for the apparent acidity of milk or as pH, tended to be in geometric progression.

In this communication evidence is submitted to show that under defined conditions the titratable acidity of a milk culture as ordinarily determined (*i.e.*, uncorrected) increases according to the geometric series with reasonable accuracy. This relationship then is used to develop a method of rating cheese starters according to their acid-producing ability in which a constant called the starter quotient, characteristic for each starter, is derived from titratable acidities.

EXPERIMENTAL

Principle. The method employed in this study depends upon the observation that in a milk culture under defined conditions titratable acidity increases in geometric progression with time, which increases in arithmetic progression over the major part of the range over which lactic acid is produced. Mathematically, this relationship between titratable acidity and time in its simplest form can be expressed by the equation:

$$a^{1/t} = r \qquad \text{(a)}$$

where a = titratable acidity
t = time at which a is determined
r = a constant and is equal to the ratio of the two adjacent members in the geometric progression.

In practice, however, it is more convenient to use the expression

$$\frac{\text{Titratable acidity at time } t_1}{\text{Titratable acidity at time } t_2} = k \qquad \text{(b)}$$

where k is a constant for a fixed time interval $t_2 - t_1$.

Method. All starters used in these studies were carried in skim milk autoclaved at 10 lb. pressure (115.5° C.) for 20 minutes. Transfers were made twice a week. A 1 per cent inoculum was propagated at 72° F. for the first 17 hours, and the starter then was stored in a refrigerator at 40° F. until the next transfer.

For the determination of the lactic acid-producing ability, starter transferred on a previous day is used. It is inoculated at the rate of 3 per cent (7.5 ml.) into 250 ml. pasteurized whole milk at 98° F., contained in a half-pint (Imperial measure) bottle fitted with a no. 8 rubber stopper. The milk then is mixed thoroughly, the time recorded, and a 9-ml. portion is titrated with 0.100 N sodium hydroxide, using phenolphthalein as indicator. The bottle then is placed in a suitable water bath at 98° F. It is convenient to

TABLE 1

Derivation of starter quotients from titratable acidities of starter cultures

Starter no.		Time in hours						
		0	1	2	3	4	5	6
43	Titratable acidity (%)	0.17	0.19	0.22	0.29	0.40	0.53	0.61
	1-hr. quotient			76	73*	76	87	
	2-hr. quotient				55	55		
CD	Titratable acidity (%)	0.17	0.19	0.22	0.27	0.34	0.45	0.56
	1-hr. quotient			82	79	76	80	
	2-hr. quotient					60	61	
SH9	Titratable acidity (%)	0.17	0.18	0.21	0.25	0.30	0.35	0.42
	1-hr. quotient			84	83	86	83	
	2-hr. quotient					72	71	
SH17	Titratable acidity (%)	0.16	0.17	0.19	0.22	0.25	0.29	0.33
	1-hr. quotient			86	87	86	88	
	2-hr. quotient					76	76	
KAS	Titratable acidity (%)	0.17	0.19	0.23	0.32	0.45	0.58	.....
	1-hr. quotient		83	72	71	77		
	2-hr. quotient				51			
EDM	Titratable acidity (%)	0.17	0.18	0.22	0.31	0.46	0.62	.....
	1-hr. quotient			71	67	74		
	2-hr. quotient				48	50		

* Values in bold face indicate those which meet the requirements of the defined quotient.

start the determination on the hour or the half-hour. Starting with the initial titration as zero hour, the milk is titrated at 1, 2, 3, 4, and 5 hours and recorded. It also must be mixed by inverting the bottle at the half-hour as well as at the hour. This is necessary to prevent the accumulation of bacteria in the upper layer of the milk due to creaming.

The lactic acid-producing ability of the starter now is calculated by dividing the first titratable acidity which is 0.23 per cent or over by the titratable acidity obtained 2 hours later, provided that the second titration does not exceed 0.56 per cent as lactic acid. The result is expressed as a simple ratio, omitting the decimal point for convenience, and is termed the starter quotient.

Properties of the starter quotient. The first important property of the starter quotient which must be established is its constancy. Representative data are presented in table 1. Corresponding to each starter, the first line gives the titratable acidities obtained by the method already described, at

TABLE 2

Reproducibility of the starter quotient

Starter 43		Starter CD	
Date	Starter quotient	Date	Starter quotient
Jan. 21	52	Jan. 21	63
Jan. 22	53	Jan. 22	62
Jan. 23	54	Jan. 23	61
Feb. 6	55	Feb. 4	56
Feb. 12	53	Feb. 27	55
Feb. 14	55	March 13	51

the hours indicated. The second lines gives the 1-hour starter quotients derived from the titratable acidities 1 hour apart. The last line gives the 2-hour quotients derived from the appropriate values 2 hours apart in the first line.

The following observations can be made by studying table 1. Both the 1-hour and the 2-hour starter quotients remain constant for a given starter regardless of the values of the two titratable acidities used for their calculation or the hours at which these determinations are made. The useful range of titratable acidities over which a constant starter quotient may be obtained is from 0.23 to 0.56 per cent as lactic acid. These relationships hold for the more active starters such as 43, EDM and KAS, as well as for the slow starters such as SH17 and SH9. In order to include a large portion of the range from 0.23 to 0.56 per cent titratable acidity and perhaps to submerge some of the slight irregularities which appear in the 1-hour quotient, the 2-hour starter quotient has been adopted as standard.

The next important question to be answered is the reproducibility of the results. Illustrative data on starters 43 and CD are summarized in table 2.

Starter 43 has been used in experimental cheese work in these laboratories and also is supplied from time to time to those requesting a cheese starter. It is carried under standardized laboratory conditions. On the whole, the starter quotient for 43 has remained unchanged over even a longer period than that shown in table 2. Starter CD was obtained from a commercial dairy, where it occasionally was used as a buttermilk starter but had not been given much care. It was selected for study because of its poor condition. It can be seen from table 2 that on consecutive days the value of the starter quotient showed satisfactory reproducibility. Over a period of 6 weeks much improvement or recovery was shown by this starter under proper care. The ability of some starters to improve is known and in the above instance was expected.

In general, the reproducibility of the starter quotient is considered satisfactory, keeping in mind the biological basis of the determination and excluding the long-term changes in the properties of starters, which really form a part of another problem.

A further characteristic property of the starter quotient is its independence of the percentage of the starter inoculum used in the milk culture. In table 3 are presented data obtained when starter 43 was inoculated at the rate of 1, 2, 3, and 4 per cent and the determination of the starter quotients carried out as described. The starter quotients, whether calculated on an hour or a 2-hour basis, show good agreement for all the levels of inoculum employed.

In summarizing the properties of the starter quotient, it may be said that it is a specific index of the acid-producing ability of the starter.

Comparative rating of starters. While no doubt there are excellent starters without pedigree used in the manufacture of Cheddar cheese, in general, they are derived from several central sources of supply. Available starters were obtained from St. Hyacinthe Dairy School, Kemptville Agricultural School, the Dairy Department of the Ontario Agricultural College, and the Dairy Department of the University of Alberta, all of which supply starters to the Cheddar cheese industry. These, together with the Dairy Research starter and one from a commercial dairy (CD), are included in table 4 in a comparative rating of their acid-producing ability by means of their starter quotients.

Each starter was transferred once when received and the starter quotient determined the next day. This is shown as the initial starter quotient in the table. The starters then were carried for 3 to 15 weeks under conditions referred to earlier; a final starter quotient at the end of the period indicated also is given in the table. In addition, starter quotients calculated from Babel's data (1) for titratable acidities at the fifth and seventh hours, with incubation at 98° F., are included for comparison. These are starters B1 to B5, and B7, the original numbers of the author being retained. Some

TABLE 3

Constancy of the starter quotient at varying percentages of inoculum (Starter 43)

Per cent inoculum		Time in hours						
		0	1	2	3	4	5	6
1	Titratable acidity (%)	0.17	0.17	0.19	0.20	0.26	0.37	0.49
	1-hr. quotient					**70***	**75**	
	2-hr. quotient						**53**	
2	Titratable acidity (%)	0.17	0.18	0.20	0.26	0.36	0.49	.....
	1-hr. quotient				**72**	**73**		
	2-hr. quotient					**53**		
3	Titratable acidity (%)	0.18	0.19	0.23	0.30	0.43	0.58	.....
	1-hr. quotient			**77**	**70**	**74**		
	2-hr. quotient				**54**	52		
4	Titratable acidity (%)	0.18	0.20	0.25	0.35	0.48	.....	.....
	1-hr. quotient			**72**	**73**			
	2-hr. quotient				**52**			

* Values in bold face indicate those which meet the requirements of the defined quotient.

starters were omitted because they did not provide titratable acidities 2 hours apart in the range 0.23 to 0.56 per cent.

Starter quotients for the 19 starters listed indicate widely varying abilities to produce lactic acid. The Canadian starters and Babel's starters are listed in separate series, in order of their acid-producing ability. The starters listed first in table 4 are the most active and would be expected to be the most satisfactory for Cheddar cheesemaking. As a matter of experience, starter 43 with a starter quotient of 52 has been found very satisfactory in making cheese over a period of several years.

For the majority of starters, the difference between the initial and the final ratings after 3 to 15 weeks is not large. In other words, with the exception of starters OACNZ and CD, a stability of the acid-producing ability is indicated.

TABLE 4

Comparative rating of starters

Starter no.	Initial starter quotient	Final starter quotient	Interval (weeks)	Starter no.	Initial starter quotient	Final starter quotient	Interval (weeks)
SH4	47	50	3	SH9	71	71	3
SH5	47	50	3	SH16	74	68	3
EDM	48	54	3	SH17	76	71	3
KAS	51	51	9				
43	52	52	15	B3*	55	.....	.....
OACNZ	55	66	4	B2	59	.....	.....
CD	63	53	10	B4	60	.....	.....
SH29	63	67	3	B1	65	.....	.....
OACH	64	70	4	B7	66	.....	.....
SH6	67	68	3	B5	78	.....	.....

* Data on B-series calculated from data of Babel (1).

DISCUSSION

In developing the procedure which finally was adopted for the determination of the starter quotient, consideration was given to those factors which would give a simple yet useful method for the evaluation of starters used in Cheddar cheesemaking. The equipment used is either ordinarily available or readily obtainable. Pasteurized milk was adopted as the medium in the test because a more uniform product of good bacteriological quality is more readily available at all times. Impairment of the acid-producing ability of some starters at cooking temperatures in cheesemaking and of most starters at slightly higher temperatures has been reported by several investigators (1, 3, 4, 5, 8, 10, 11). In fact, high cooking temperature is a standard method of retarding too rapid acid development in the vat. For this reason and because a good starter must be able to withstand at least this temperature during cooking, 98° F. was selected for the determination of the starter quotient.

With respect to the starter quotient itself, it already has been pointed out that it is constant over the major portion of the range over which lactic acid is produced, and is characteristic of a given starter. Furthermore, it is independent of the amount of inoculum used and allows a considerable latitude with respect to the choice of time or titratable acidities which may be used for its calculation. This makes it possible to make comparisons on an absolute basis of starter quotients determined in different laboratories under various conditions. A 3 per cent starter inoculum has been selected for our determination since it allows a determination to be carried out conveniently during the working day, and still gives good results. However, should it be necessary, a different percentage of inoculum may be used. The starter quotient is independent of the initial stationary and lag phases and the final inactive and death phases of bacterial growth. Much of the uncertainty which characterizes these phases and is included in a determination of the over-all growth rate therefore is eliminated from the starter quotient. Finally, from a comparison of acidity-time curves such as those of Monrad and Helmer (6) for milk and the ones given by Dolby (4) for cheese curd, it appears that the rates of acid development in milk and in cheese curd are not dissimilar. To this extent, therefore, a rating of a starter in milk culture also should be an index of its behavior in the cheese curd.

Only brief comment is offered on the question of variability of the starter quotient. The cheese starters in common use on this continent are made up of several species of lactic acid-producing organisms. An equilibrium among these organisms is maintained by uniformity of the conditions in carrying the starter. In addition, there is a variability of properties of a normal population of each species or each strain. Equilibria between the acid tolerant and non-tolerant organisms and between temperature tolerant and non-tolerant organisms would be important. The starter quotient, therefore, is an index of the average acid-producing ability of all the organisms present in the starter. While a starter may, over a period of time, become unsuitable for cheesemaking from the point of view of acid production, this is not a weakness of the starter quotient; on the contrary, the starter quotient may be used in ascertaining when the starter needs attention or replacement.

SUMMARY

Starter which has been transferred the day before is inoculated at the rate of 3 per cent into 250 ml. pasteurized whole milk and incubated at 98° F. Titrations are made at 0, 1, 2, 3, 4, and 5 hours, and, in addition, the milk cultures are mixed every half hour. The lactic acid-producing ability of the starter is calculated by dividing the first titratable acidity which is 0.23 per cent or over by the titratable acidity obtained 2 hours later, provided that the second titration does not exceed 0.56 per cent. The result is expressed as a simple ratio, omitting the decimal point for convenience, and

is termed the starter quotient. Mathematically, the starter quotient is based upon the observation that titratable acidity in a milk culture under defined conditions increases geometrically. The quotient remains constant over the range 0.23 to 0.56 per cent and is independent of the amount of inoculum used. Data are presented illustrating the properties of the starter quotient, and a comparative rating of 13 Canadian cheese starters is given.

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OBSERVATIONS ON THE INSTABILITY OF THE PROTEIN PHASE OF FROZEN CONCENTRATED MILK[1]

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The gradual progressive flocculation of some of the protein of milk as a result of frozen storage has been observed by a number of workers, some of whom (1, 2, 3, 5, 6, 8, 9, 11, 14) have noted that the instability of the protein phase is aggravated by such influences as lengthened periods of storage, comparatively high storage temperatures, increased concentration of the milk, and comparatively high heat treatments applied as forewarming or superheating procedures in advance of freezing. It also has been noted that the flocculation is reversible with heat to a remarkable degree (5, 8, 9, 14), but factors affecting reversibility have not been studied. Little is known concerning the effect of "stabilizing salts" or of higher pH in delaying this type of protein precipitation (4, 10, 11), although with other types of coagulation they are known to be inhibiting.

The causes of protein flocculation in frozen stored milk are not established definitely, but they are best postulated by Sommer (13), who suggests that higher salt concentration, probable higher acidity, physical crowding of the protein micelles by ice crystals, and loss of water of hydration are the destabilizing factors, with the increasing viscosity as an opposing factor. Webb and Hall (14) are convinced that calcium caseinate (a weakly hydrated sol in milk) comprises the bulk of the flocculated protein and believe that frozen storage further lessens the hydrophylic character of this protein so that on thawing it behaves more as a strictly hydrophobic substance.

Existing knowledge concerning the flocculation phenomenon still is meager and in several respects inadequate. In view of the likelihood that more milk and milk products will be stored and handled in the frozen state as the possibilities become better appreciated, it seemed desirable to acquire more information concerning the instability of the protein phase, particularly since this appears to be the limiting factor in the storage life of frozen concentrated forms of milk.

EXPERIMENTAL METHODS

Inasmuch as protein flocculation during frozen storage was the only phenomenon under study, all observations were made on skimmed milk to eliminate the interfering effect of the fat phase. Fresh, factory-skimmed milk was forewarmed, concentrated, cooled, standardized, filled into suitable

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pint containers, and frozen statically at approximately – 10° F. (– 23.3° C.). After 24 hours, when solidly frozen, it was stored at either + 5° F. (– 15° C.) or at – 10 to – 15° F. (– 23.3 to – 26.1° C.). A sufficient number of pint containers of each lot of milk was filled to allow periodic withdrawal of one at each observation during the course of frozen storage. The higher storage temperature used was obtained in a large, accurately controlled ice cream cabinet, and the lower in an experimental hardening room which fluctuated about 5° F. (2.8° C.), although in a few comparisons the fluctuation amounted to 10° F. (5.6° C.).

When examined, frozen samples were taken from the containers, weighed into 2-l. beakers and reconstituted and thawed with the proper weight of distilled water at 180° F. (82.2° C.). Defrosting was accomplished without agitation and the appearance of the thawed samples was noted. They then were thoroughly agitated by pouring ten times.

Volume of Flocculated Protein

A 50-ml. portion of the well-mixed sample was placed in a graduated, tapered, centrifuge tube and whirled at 2,000 r.p.m. for 15 minutes in a centrifuge with a 12-inch head. The tubes were decanted carefully, made up to the original volume with ice-cold distilled water, centrifuged again for 10 minutes, and the per cent of flocculated protein measured by volume. This method is similar to that of Anderson and Pierce (1), except that they weighed the protein in a wet state, and is almost identical with that of Babcock *et al.* (2), who reported results as "cc. of sediment".

Other Tests

Alcohol numbers were determined as described by Doan (7). Titratable acidity measurements followed the recommendation of Sommer (12). Determinations of pH were made at 25° C. with a Beckman industrial model pH meter, utilizing a glass electrode standardized by means of buffer tablets made by Coleman Instrument, Inc., according to the formulas of Kolthoff, McIlvaine, and others, using sodium tetraborate and potassium phosphate.

Heat Redispersion of Flocculated Protein

About 100 ml. of a well-mixed defrosted and reconstituted sample were placed in a 300-ml. beaker and heated over a low flame with continuous agitation until a thermometer in the liquid registered exactly 150° F. (65.5° C.) or 200° F. (93.3° C.), depending on the degree of flocculation present, after which the beaker immediately was immersed in ice water. After heat treatment, the volume of flocculated protein again was determined as a measure of the degree of redispersion accomplished by the heat treatment.

Other methods and variations employed are described in the appropriate places.

EXPERIMENTAL RESULTS

The appearance of variously treated samples of concentrated skim milk after 12 weeks of frozen storage is illustrated in figure 1. These samples were defrosted and reconstituted without agitation. They exhibit various abilities to disperse into the water of reconstitution, depending on the individual degree of protein denaturation. Figure 2 shows two of the samples, of differing dispersibilities, after pouring ten times and standing 10 minutes. It will be noted that sample no. 2 presented an entirely normal appearance after agitation, whereas no. 4 showed a definite tendency to settle on the part

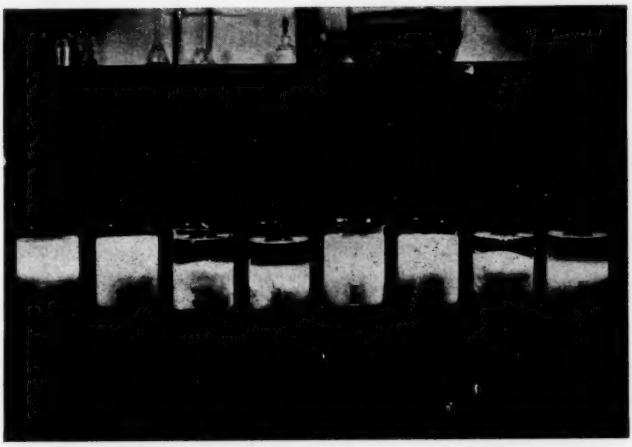

FIG. 1. A series of samples of thawed and reconstituted frozen concentrated skim milk after 12 weeks storage. (Samples 1 to 4, forewarmed at 190° F. for 15 minutes; 5 to 8 at 190° F., flash. Samples 1, 2, 5, and 6 stored at −10 to −15° F.; 3, 4, 7, and 8 at +5° F. Samples 1, 3, 5, and 7 reconstituted with water at 80° F.; 2, 4, 6, and 8 with water at 180° F.)

of the small flakes present. When the no. 4 sample was heated to 150° F., however, it became just as normal in appearance as no. 2.

Nature of the Floc

Characteristics. The protein flocculation gradually occurring during the frozen storage of concentrated milk is not apparent while the milk is frozen. On thawing, and particularly on thawing accompanied by dilution with water, it first becomes evident as a fine, feathery, and somewhat fragile precipitate. Initially, it seems to redisperse on mere standing. When more pronounced, it can be dispersed by vigorous agitation, but eventually denaturation proceeds so far that no amount of simple agitation will cause it to assume a normal dispersed condition. In advanced stages, a gross precipitation and a wheying-off are evident.

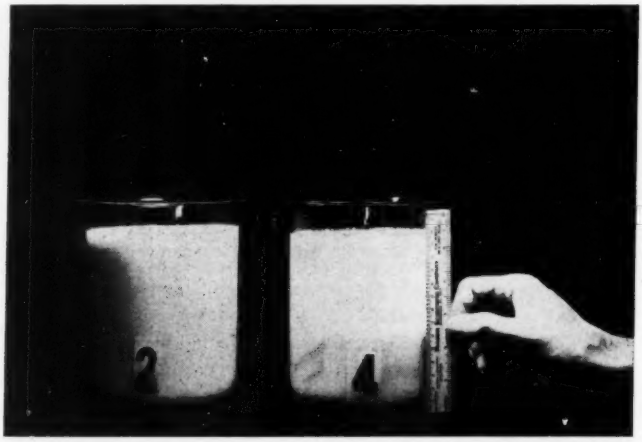

FIG. 2. Two of the samples shown in figure 1 after pouring ten times and standing 10 minutes. (Sample 2 stored at – 10 to – 15° F. Sample 4 stored at + 5° F.)

Freezing alone causes no measurable precipitation of the protein. This was noted scores of times during the course of the study. Measurable amounts of floc were obtained only after a time interval in the frozen state, the length of the interval being governed by other conditions. Typical results are shown in table 1. No significant deposits of protein were obtained on centrifugation of these samples until after 1 week of storage at + 5° F., or until after 8 weeks of storage at – 10 to – 15° F.

The relative fragility of the floc and its tendency to redisperse in the

TABLE 1

The volume of flocculated protein produced by frozen storage of concentrated skim milk (4:1)

(Reconstituted basis)

Weeks in storage	Immediately after thawing and mixing		2 hr. at room temperature after thawing and mixing	
	At – 10 to – 15° F.	At + 5° F.	At – 10 to – 15° F.	At + 5° F.
	(%)	(%)	(%)	(%)
Unfrozen	0.50	0.50	0.50	0.50
0	0.50	0.50	0.50	0.50
1	0.50	8.00	0.50	2.50
2	0.50	15.00	0.50	7.00
4	0.75	15.50	0.50	15.00
8	1.00*	15.00	0.75	16.00
10	2.50	15.00	0.75	18.00
12	8.00	15.00	0.75	17.50

* Visual evidence of flocculation in thawed reconstituted samples is noted between 1.00 and 2.00 per cent.

early stages are well shown by a comparison of the volumes obtained immediately after thawing and after 2 hours of standing. In the later stages, redispersion is not evident; in fact, the tendency is toward greater volume of precipitate, which may be due to gradual hydration insufficient to cause peptization but enough to increase the bulk of the deposit in the centrifuge tube. It is believed that this tendency to redisperse on standing is responsible for some of the minor fluctuations noted in the data on volume of precipitate throughout the study.

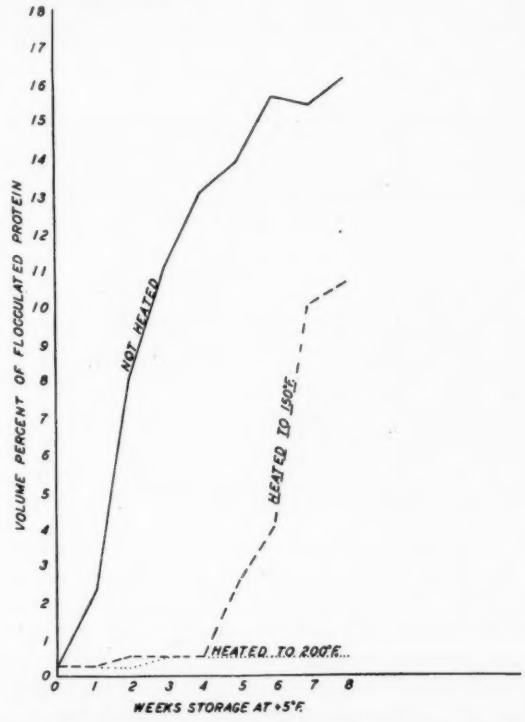

FIG. 3. Redispersion by heat of flocculated protein in frozen and stored concentrated skim milk (3:1) (average of 13 trials).

Reversibility with heat and agitation. The effect of heat in peptizing the flocculated protein of thawed and reconstituted frozen milk after various intervals of frozen storage is strikingly shown in figure 3. After 8 weeks of storage, the samples exhibited an average of about 16 per cent flocculation. When heated to 150° F., they showed only about 10 per cent and when heated to 200° F., the volume of flocculation was insignificant. Agitation and homogenization alone or coupled with heat also causes a redispersion of

frozen storage flocculated protein, as shown by the data in table 2. It appears, therefore, that the redispersion of this protein is in the nature of a simple dissociation accomplishable by heat or mechanical work.

Similarity to "salted-out casein". Observations on the protein flocculation encountered in this study led to the belief that the precipitation is in the nature of a "salting out" process, readily reversible in the early stages merely by removing the cause but becoming less reversible with time. The effect of the time factor on the properties of a "salted-out" calcium caseinate held in the brine at room temperature was compared with the properties previously presented of a protein flocculated under conditions of frozen storage.

TABLE 2

Redispersion of protein flocculated in milk by frozen storage as influenced by heat and mechanical work

Treatment of thawed reconstituted milk	Volume of flocculated protein	
	Stored 4 weeks	Stored 8 weeks
	(%)	(%)
Immediately after mixing	13.00	16.50
Standing 2 hr. after mixing	9.00	18.00
After mixing and heating to 150° F.	1.00	10.50
After mixing and heating to 200° F.	0.75	3.00
After mixing and homogenizing* at 70° F.	4.00	11.50
After mixing and homogenizing* at 150° F.	0.50	1.50
After mixing and homogenizing* at 200° F.	0.50	0.75
After mixing and agitating† at 70° F.	3.00	10.00
After mixing and agitating† at 150° F.	0.75	1.00
After mixing and agitating† at 200° F.	0.50	1.00

* Accomplished by means of a laboratory hand homogenizer.
† Accomplished with a Waring Blendor in 60 seconds.

A portion of concentrated skim milk (3:1) was treated in a large Erlenmeyer flask with 36 g. of sodium chloride per 100 g. of water present and allowed to stand at room temperature. At intervals the precipitated mixture was agitated thoroughly and two samples removed for examination. The first was simply reconstituted, resulting in a dispersion which was about one-fourth saturated with sodium chloride. A portion of this was allowed to stand at room temperature for 2 hours while two other portions were heated to 150 and 200° F., respectively. The second sample was dialyzed against cold tap water for 48 hours followed by reconstitution, and a portion of this was heated to 200° F. The volume of the precipitate then was determined on all portions of each of the samples in the usual manner. The results are given in table 3.

The development and behavior of salted-out calcium caseinate stored in the brine and of protein precipitated from milk by frozen storage are, to all intents and purposes, identical. At first, both are reversible with removal of the cause, but upon introduction of a time factor, reversibility gradually

is lost. Both are reversible for a time by heating, but eventually this too becomes ineffective.

Some of the dialyzed calcium caseinate salted from skim milk was compared with the washed coagulum obtained from some of the frozen and stored concentrated milk samples after dispersing by reconstituting and heating. Treatment with acids, alkalies, salts of heavy metals and alcohol, saturation with sodium chloride and magnesium sulfate, heating to boiling, and additions of rennin and rennin plus calcium chloride gave identical results. The sols from each type of precipitate gave similar isoelectric ranges (pH 4.60 to 4.72), as judged by the turbidity of filtrates from series tests made by adding increasing amounts of acetic acid. It seems justifiable to conclude

TABLE 3

Volume and behavior of salted-out calcium caseinate when stored in the brine at room temperature in the form of concentrated skim milk (3:1) saturated with sodium chloride

Stored at room temperature	Volume of flocculated protein					
	Undialyzed but reconstituted (about ¼ saturated with NaCl)				Dialyzed 48 hr. and reconstituted	
	At once	After 2 hr. standing	Heated—150° F.	Heated—200° F.	Cold	Heated—200° F.
(*weeks*)	(%)	(%)	(%)	(%)	(%)	(%)
0	0.25	.....	.....	.....	.....	.....
1	3.00	0.50	0.25	0.25	0.25	.....
2	4.00	2.00	0.25	0.25	0.25	.....
4	7.50	3.50	0.25	0.25	0.25	.....
6	10.00	8.00	1.00	0.25	0.25	.....
8	22.00	10.00	2.50	0.75	1.00	0.25
12	24.00	18.00	7.50	3.00	26.00	2.00
14	24.00	26.00	20.00	17.00	28.00	3.00
18	22.00	23.00	24.00	22.00	34.00	6.00
22	24.00	26.00	24.00	24.00	31.00	24.00

that the flocculation developing in frozen and stored milk is calcium caseinate, as Webb and Hall (14) have assumed. Only traces of albuminous substances were detectable in the prepared sols from frozen milk flocs, indicating that these were in the nature of contaminants and not a part of the coagulum proper.

The Effect of Freezing and Frozen Storage of Concentrated Skim Milk on Titratable Acidity, pH, and Alcohol Number

Determinations of titratable acidity, pH, and alcohol number (protein stability) were made on a large number of skim milk samples variously treated, concentrated, frozen, and stored. These were made before freezing, after freezing, and at various intervals during frozen storage. No significant changes in any of these values with any of the samples were noted when

comparisons were made on an equivalent reconstituted basis. Alcohol numbers, of course, could not be obtained on stored samples which exhibited visible flocculation. Nevertheless, when the protein of such samples was redispersed by heating, the alcohol number showed no change from the original unfrozen sample. It therefore can be concluded that neither freezing nor freezing and storage causes any permanent change in these three characteristics of milk.

Effect of Forewarming Temperature of the Concentrated Skim Milk on Protein Stability in Frozen Storage

Preliminary comparisons showed that the interval of heating fluid skim milk prior to condensing exercised an insignificant influence on the stability of the protein in subsequent frozen storage but that the temperature used had considerable effect. Several trials were made using forewarming temperatures from 145 to 208° F. (62.8 to 97.7° C.). Typical results are presented in table 4 for samples stored at −5 to −15° F. Similar results were obtained at +5° F., but the flocculation was much more rapid and the effect of the variable was less clear.

TABLE 4

The volume of flocculated protein in frozen and stored concentrated skim milk (3:1) as influenced by the forewarming temperature
(Storage at −5 to −15° F.)

Weeks in storage	Temperature of forewarming (approx. 15 min.)					
	145° F.	160° F.	170° F.	180° F.	190° F.	200° F.
	(%)	(%)	(%)	(%)	(%)	(%)
Unfrozen	0.50	0.50	0.50	0.50	0.50	0.50
0	0.50	0.50	0.50	0.50	0.50	0.50
2	0.50	0.50	0.50	0.75	0.50	0.75
4	0.50	0.50	0.50	0.75	0.75	0.75
6	1.00	0.50	0.50	1.00	0.75	0.75
8	1.00	0.75	0.50	1.50	2.00	1.50
10	1.00	0.75	0.75	5.25	5.50	6.50
12	1.25	0.75	0.75	11.00	7.25	9.00
14	1.25	1.00	0.75	11.50	9.00	13.75
16	1.50	1.00	0.75	13.50	12.50	13.75

Temperatures of forewarming between 145° F. and the apparently critical point of 170° F. do not influence materially the rate of flocculation, but with higher temperatures the stability of the protein definitely is lessened.

Effect of Concentration of Milk and Storage Temperature on Protein Stability in Frozen Storage

Increasing concentration of the milk and increasing storage temperatures previously have been reported as decreasing the stability of the milk protein during frozen storage. The results obtained in this study are in accord with

previous results and are tabulated in table 5. These are averages of six trials at various forewarming temperatures using paired samples.

Effect of Added Salts on Protein Stability in Frozen Storage

A large batch of concentrated skim milk was divided into six parts. Each of five parts received additions of one of the following salts, dissolved in the water of standardization: calcium acetate, sodium citrate, disodium phosphate, sodium hexametaphosphate, and sodium tetraphosphate. The amounts added were sufficient to produce a M/600 concentration of the respective salt on the basis of the volume of milk. This was repeated with concentrations of M/300 and M/150. The variously treated samples and

TABLE 5

The volume of flocculated protein in frozen and stored concentrated skim milk as influenced by concentration and storage temperature (average of six trials)

Weeks in storage	At −5 to −15° F.		At +5° F.	
	Concentrated 3:1	Concentrated 4:1	Concentrated 3:1	Concentrated 4:1
Unfrozen	0.25	0.50	0.25	0.50
0	0.25	0.50	0.25	0.50
2	0.50	0.50	8.00	10.50
4	0.50	0.75	11.25	15.00
6	0.50	1.00	15.00	14.00
8	1.00	1.50	16.25	14.00
10	2.50	4.25	.....	.....
12	4.25	5.75	.....	.....
14	5.75	8.00	17.00	16.00
16	6.50	14.00	.....	.....

a control then were frozen, stored at +5° F. to accelerate the action and examined at intervals as usual.

Protein flocculation was not affected by calcium acetate, was slightly retarded by sodium citrate, but was considerably inhibited by the calcium sequestering salts (polyphosphates) which contributed a slightly objectionable astringent flavor at the highest concentration used. The results of this study with the M/150 concentrations are shown graphically in figure 4.

If, as has been suggested, protein flocculation in frozen storage is due to a "salting out" effect, it is difficult to visualize how relatively slight additions of salts of the type used would exercise any material influence on the stability of the milk protein because the water of the milk must be at or near the saturation point of several naturally-occurring salts. It appears, however, that the suppression of the calcium ion is an effective stabilizing factor even under these extreme conditions.

Effect of pH Adjustment on Protein Stability in Frozen Storage

Two trials were made in which the pH of the concentrated skim milk was increased by 0.4 and decreased about 0.4 by means of sodium hydroxide

and lactic acid, respectively, added in the water of standardization. The results of the two trials were almost identical with respect to the effects on the development of the protein flocculate in frozen storage and on its ability to be redispersed with heat. The experience with one trial is presented in table 6.

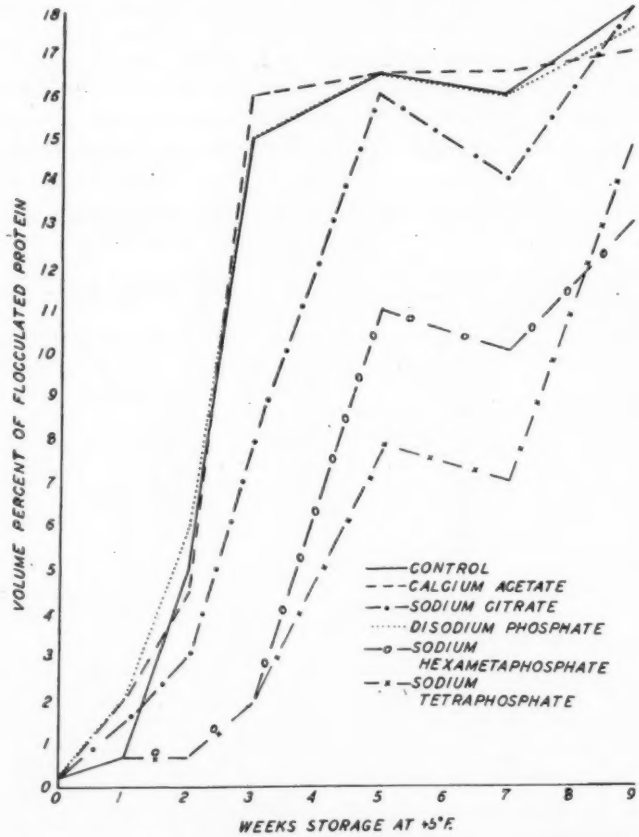

FIG. 4. The volume of flocculated protein in frozen and stored concentrated skim milk (3:1) as influenced by additions of various salts in M/150 concentrations.

Either increasing or decreasing the pH prior to the frozen storage of milk caused a slight but hardly significant acceleration of the rate of flocculation. Heat reversibility of the precipitation, however, very definitely was hindered by acidification and was favored slightly by alkalization. These results point to the importance of avoiding the use of high acid milk or milk

which has undergone significant bacterial action when a product possessing qualities of long storage life is desired.

SUMMARY AND CONCLUSIONS

Freezing, in itself, produces no measurable flocculation of the protein of concentrated skim milk which is not immediately reversible on defrosting and reconstitution. Frozen storage, however, causes a gradual change in the protein properties whereby, with time, the reversibility is lessened and a floc can be separated and washed by centrifugation. In the early stages

TABLE 6

The volume of flocculated protein in frozen and stored concentrated skim milk (3:1) and its ability to be redispersed by heat as influenced by pH adjustments prior to freezing and storage at +5° F.

Concentrated milk before freezing { Control—pH = 6.40; Alkalized—pH = 6.79; Acidified—pH = 6.02

Weeks in storage	Control—reconstituted pH = 6.62			Alkalized—reconstituted pH = 7.00			Acidified—reconstituted pH = 6.30		
	Cold	Heated—150° F.	Heated—200° F.	Cold	Heated—150° F.	Heated—200° F.	Cold	Heated—150° F.	Heated—200° F.
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Unfrozen	0.25	.....	.....	0.25	.....	.....	0.25	0.25	.....
0	0.25	.....	.....	0.25	.....	.....	0.25	0.25	.....
1	0.50	0.25	0.25	2.50	0.25	0.25	1.50	0.75	1.00
2	15.50	0.25	0.25	20.00	0.25	0.25	17.00	2.50	4.00
3	15.00	0.25	0.25	16.50	0.25	0.25	16.00	10.00	10.00
4	15.00	0.25	0.25	16.50	0.25	0.25	15.50	14.00	11.00
5	16.50	0.25	0.25	18.50	0.25	0.25	16.00	13.00	12.00
7	15.50	1.00	0.50	17.50	0.50	0.25	15.00	14.00	11.00
9	18.00	1.00	0.50	20.00	0.50	0.25	17.00	14.00	14.00
12	19.00	2.00	0.75	19.00	0.50	0.25	17.50	15.00	15.00

this floc is redispersible with time or immediately by heat or agitation. In extended frozen storage the precipitated protein eventually loses completely its reversible character.

Calcium caseinate "salted-out" of milk exhibits identical properties and behavior, when stored at ordinary temperatures in the brine, to those of the flocculated protein separated from milk after frozen storage. Qualitative tests of the two precipitates also are identical. It is concluded, therefore, that the floc of frozen milk is calcium caseinate which has been thrown out of its normal colloidal dispersion by a "salting out" action of the high concentration of milk salts in the unfrozen water of the product.

Neither freezing concentrated skim milk nor holding it in the frozen state causes any change in titratable acidity, pH, or alcohol stability when determined after defrosting and reconstitution. Whatever alterations may exist in the frozen state, they are reversed quickly on defrosting.

As has been noted previously, flocculation of the casein is accelerated by

concentration of the milk, higher storage temperatures, and longer periods of storage. It also is accelerated by the use of temperatures over 170° F. (76.6° C.) when applied as forewarming treatments in the process of concentrating the milk. Temperatures up to this point do not seem to cause any significant variations.

Calcium acetate and disodium phosphate in concentrations up to M/150 do not affect the rate of flocculation. Sodium citrate slightly decreases it, and the calcium-sequestering polyphosphates definitely retard it but introduce a detectable astringent flavor.

Neither raising nor lowering the pH of concentrated skim milk prior to frozen storage significantly alters the rate of casein flocculation. Both accelerate it very slightly. However, the reversibility of the coagulation with heat is decreased decidedly when the milk is acidified and is increased slightly when alkalized. This emphasizes the importance of avoiding bacterial action in milk to be frozen and stored commercially.

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PROPERTIES OF THE COLOSTRUM OF THE DAIRY COW. I. TOCOPHEROL LEVELS IN THE COLOSTRUM AND IN THE EARLY MILK[1]

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The elucidation of the role of colostrum in the nutrition of the dairy calf suggested a study of the tocopherol concentration in this secretion. Such an investigation has received little attention. Mason (4) and Mason and Bryan (5) investigated this problem in rats by means of bioassays. They found that tissues of suckling rats from 24 to 48 hours of age were three to four times higher in vitamin E than were those of the newborn, indicating that colostrum of the rat was a rich source of tocopherols. Quaife (7), using a chemical procedure, found that early human milk had a higher tocopherol content than later milk.

In an investigation of the nutritional significance of vitamins A and E for the dairy cow, an opportunity was presented for studying the tocopherol content of the mammary secretions during the period of transition from colostrum to normal milk. The results of this phase of the investigation are reported herein.

EXPERIMENTAL

Grouping, feeding, and management of experimental animals. Dairy cows and heifers of four breeds, Holstein, Ayrshire, Jersey, and Guernsey, were grouped on the basis of the quantity of supplemental tocopherols added to the ration (table 1) and according to the management of the cows. Group I included all the animals that did not receive supplemental tocopherols[3] and were subjected to standard herd management. Since a survey of the data, as indicated in subsequent results, revealed no marked effect of vitamin A[4] supplementation on tocopherol concentrations in colostrum, the cows supplemented with vitamin A only were included in this group. Group II received vitamin A in addition to tocopherols; Group III received only tocopherol supplements. The cow in group IV received no supplemental vitamins and was milked throughout the gestation period. Details of the

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[3] Tocopherols in a soybean flour base were supplied by Distillation Products, Inc., Rochester, N. Y.

[4] Three forms of vitamin A concentrates were used: (a) vitamin A alcohol in a soybean flour base, (b) vitamin A alcohol in an oil medium, and (c) natural vitamin A ester in an oil medium. The first product was supplied by Distillation Products, Inc., Rochester, N. Y., and the latter two by the Borden Co., New York, N. Y.

quantities of vitamins given and the periods of supplementation are presented in table 1.

Vitamin A was included in this investigation, since the data presented herein were supplementary to a basic study of the effects of tocopherols on vitamin A and carotene in dairy cattle. All these supplements, except tocopherols in excess of 1 g., were administered *per os* in gelatin capsules; the larger amounts of tocopherol were combined with the concentrate mixture immediately before feeding. When both vitamins A and E were given, they were premixed and administered in the same capsule. Supplementation was discontinued at parturition. The rations were consumed readily by all animals except no. A–200, which refused some concentrate feed when the tocopherol supplement was introduced; however, the scheduled level of intake was resumed about 2 weeks before calving.

TABLE 1

Supplements given to pregnant cows daily

Group	Sub-group	No. of animals	Days previous to date of expected parturition	
			28–14	14–0
I	a	5	No supplement	No supplement
	b	6	500,000 I.U. vit. A alc.	1,000,000 I.U. vit. A alc.
	c	7	500,000 I.U. vit. A ester	1,000,000 I.U. vit. A ester
II	.....	6	500,000 I.U. vit. A alc. and 0.5 g. α–γ-tocopherol	1,000,000 I.U. vit. A alc. and 1 g. α–γ-tocopherol
III	a	3	10 g. mixed tocopherol	10 g. mixed tocopherol
	b	1 (G–451)	0.5 g. α–γ-tocopherol	1 g. α–γ-tocopherol
	c	1 (G–467)	4 g. mixed tocopherol	4 g. mixed tocopherol
	d	1 (A–240)	5 g. α–γ-tocopherol	5 g. α–γ-tocopherol
IV	.....	1 (H–126)*	No supplement	No supplement

* Cow milked through gestation, without the dry-rest period.

The experimental subjects, except cow H–126, which was maintained on winter milking-herd rations, were fed a standard control diet consisting of a concentrate mixture (12.5 per cent crude protein), a good quality Atlas Sorgo silage, and high grade alfalfa hay. The concentrate feed was given at the rate of 1 lb. daily for 100 lb. of body weight, using the initial weight of the cow at the start of the trial as the basis for all subsequent feeding, which extended 7 days postpartum. Hay and silage were fed in the ratio of 1 : 2, the quantity being adjusted to the appetite of the individual cow. The animals were placed on the standard control ration approximately 6 weeks prepartal; the respective vitamin supplements were introduced into the diet 4 weeks before the date of expected parturition. Silage was fed in the morning, hay in the afternoon, and concentrates in equal portions at the morning and evening feeding periods. Whenever the weather permit-

ted, the cows were released into an open lot, where water, salt, and bone meal were accessible.

Collection of colostrum and milk samples. It is recognized that as the milk is removed from the udder the fat content increases several-fold from the foremilk to the strippings. Since a similar increase has been observed for colostrum (6), the use of small samples collected directly from the udder may give an erroneous conception of the concentration of fat-soluble substances which apparently include the tocopherols. Therefore, special precautions were taken to obtain samples that were representative of the entire mammary secretions at various stages.

To prevent removal of colostrum by the newborn calf, the udder of the parturient cow was covered. The calf was segregated from its dam within 4 hours after birth and was not permitted to nurse at any time. A further measure to insure representative sampling was emptying the udder as completely as possible by standard milking procedures, including stripping. (This, contrary to popular belief, apparently did not predispose the dams to milk fever.) The mammary product, colostrum or milk, was mixed thoroughly before aliquots were taken for analysis. The first colostrum was obtained usually less than 2 hours after calving and always within 6 hours. Subsequent samples were taken at the regular morning and evening milking. The first four collections were analyzed individually, and the following collections, on the third, fourth, and eighth days, were analyzed as daily composites.

Analytical procedures. The method for determining the tocopherol concentration differed from that used by Quaife (7) only in the following respects: The samples for analysis ranged from 10 to 30 ml., depending upon the concentration of tocopherols. The removal of the Skellysolve B to obtain the residue containing the tocopherols was accomplished by evaporation under suction, using a water bath at 70° C. for heating. A Coleman spectrophotometer with a special cell holder that accommodated $7 \times \frac{1}{8}$ inch absorption tubes was used in the assay. Readings were made at a wave length setting of 520 mμ. The instrument was calibrated using pure natural α-tocopherol.[5]

Since colostrum often is high in carotenoid pigments, care was taken to decolorize the extract completely by hydrogenation; sometimes it was necessary to use smaller aliquots to accomplish this. Occasionally fats tended to separate from the final hydrogenated extract. This precipitated material redissolved if the absorption tube containing the extract was placed in a water bath at 35° C. and shaken gently prior to the addition of the analytical reagents.

Milk fat content was determined by the standard Babcock procedure.

Method of expressing results. Not only was considerable variation found

[5] Supplied by M. L. Quaife, Distillation Products, Inc., Rochester, N. Y.

in the fat content of the mammary secretions from different cows during the colostric period but also from milking to milking in the same individual. To illustrate the possible effect of this latter factor upon the results when considering either a single or a few individuals, the tocopherol data for a typical group (I–c) and for an individual (G–468) which shows considerable deviation from the group average, are expressed in both γ/100 ml. of fluid and γ/g. of fat (fig. 1). The group averages (similar to those of the other experimental groups but not shown) indicate that the normal tendency is for tocopherol concentration to change from milking to milking in a definite manner. This decrease, as indicated in the subsequent discussion,

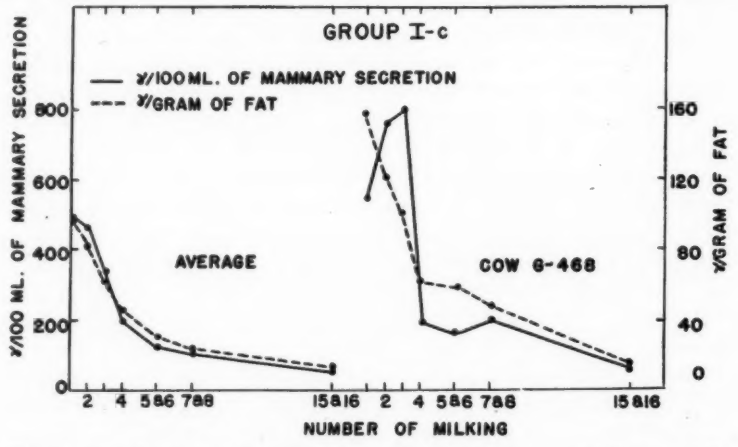

FIG. 1. Tocopherols in colostrum and in milk expressed as concentration per unit of fat and as concentration per unit volume.

has been found to follow the exponential law (fig. 2). The graph for cow G–468 (fig. 1) illustrates how the seemingly natural trend, as judged from the average, sometimes may be obscured unless tocopherol concentration is related to fat content. Consequently, tocopherol values are expressed herein as concentration per unit of fat.

RESULTS AND DISCUSSION

An examination of the results, shown in detail in table 2, reveals that there was considerable individual variation in the tocopherol content of the mammary secretions of cows within the same dietary group. The limited number of observations did not indicate any appreciable differences ascribable either to the breed of cow or to the number of previous lactations. However, additional data will be required to establish fully the influence of these aforementioned factors. While the first sample of colostrum usually

had the highest tocopherol content per unit of fat, the maximum value for several cows was reached in the second milking.

The data of table 2 indicate that within group I, the average tocopherol content of the colostrum removed in the first milking was the highest for subgroup b and lowest for subgroup a, the average of the latter being almost the same as that for subgroup c. The average values for each of the three subsequent milkings were lowest for subgroup c; but as a whole, no striking differences are evident when values for the various samples from each of the three subgroups are compared. Apparently the addition of vitamin A to the basal diet had no definite effect on the level of tocopherols in the colostrum.

Tocopherol concentration in early colostrum was influenced by the levels of tocopherol intake (table 2 and fig. 2). Supplementation with 0.5–1 g. of tocopherols (group II) increased the concentration of this substance in colostrum 1.4 times the average level for cows not receiving this vitamin (group I). When 10 g. of tocopherols were given daily (group III–a), the concentration of these substances in first colostrum was 4.5 times that found for unsupplemented cows (group I). The ratios of the tocopherol levels in the first sample of colostrum of the foregoing groups of cows were essentially the same as those for blood serum tocopherol levels of the respective groups at the time of parturition (3).

There was a tendency for cows supplemented at the higher levels to maintain a greater concentration of tocopherols in the mammary secretions over a longer period of time than did those having a lower intake. Cow A–200 (table 2, group III–a), which refused to consume all the supplement in the early stages, was an exception to the general trend.

The low tocopherol values for H–126, which was milked throughout the gestation period, are in sharp contrast to those having the rest period. This suggests that normally within this period the cow accumulates reserves of tocopherols which rapidly are eliminated during the colostric period to provide for specific needs of the calf. However, the tocopherol requirements, which have not been established for the calf, may be less than the quantity normally obtained through ingestion of colostrum.

No data on the tocopherol content of silage have been found, but the grains and hay of the daily basal ration fed the cows probably contained from 0.5 to 1.4 g. of tocopherols, based upon the limited data of Cabell and Ellis (1). Presumably on a basal diet of lower tocopherol content the difference in tocopherol levels of colostrum from supplemented and unsupplemented animals would be greater than observed in this study.

In comparing the tocopherol concentration of the first mammary secretion with that of the eighth day, it was found that for cows receiving no supplemental tocopherols (group I), the average level of these substances was seven times higher in the first secretion than in the latter. Similarly,

TABLE 2

Tocopherol concentration in the fat of colostrum and of milk at different stages in the transformation period

Group	Subgroup*	Ration supplement	No. of lactation	No. of milking						
				1	2	3	4	5 & 6	7 & 8	15 & 16
				Tocopherols, γ/g. of fat						
I	a									
	H–175	None	6	94	77	71	57	50	25	24
	H–105		4	80	94	74	62	40	22	16
	A–230		3	107	117	70	.....	53	29	17
	G–433		5	109	100	76	62	36	17	16
	G–432		5	101	98	76	63	26	46	26
	Subgroup mean			98	97	73	51	41	28	20
	b									
	H–115	Vitamin A alc.	2	52	46	34	25	16	12	11
	A–252		1	151	136	115	74	38	40	27
	A–243		2	111	85	84	65	41	25	10
	J–365		2	175	87	75	48	30	21	11
	G–465		1	148	139	65	54	28	14	.....
	G–452		2	93	82	56	40	30	15	16
	Subgroup mean			122	96	72	51	31	21	15
	c									
	H–132	Vitamin A ester	1	103	84	55	35	29	.....	.....
	H–128		2	91	78	48	42	21	22	12
	A–241		2	73	63	50	27	23	19	12
	J–378		1	64	79	66	57	42	24	18
	J–358		2	136	108	78	68	43	22	8
	G–468		1	157	122	102	63	59	48	14
	G–453		2	73	54	37	15	16	12	13
	Subgroup mean			100	84	62	44	33	25	13
Group mean				107	92	69	52	35	25	16

TABLE 2—(*Continued*)

Group	Subgroup*	Ration supplement	No. of lactation	No. of milking						
				1	2	3	4	5 & 6	7 & 8	15 & 16
				Tocopherols, γ/g. of fat						
II	H–135	Vitamin A	1	152	143	123	58	36	26	16
	H–136	alc. and	1	150	114	90	65	44	26	16
	J–374	0.5–1 g.	1	143	144	106	89	54	41	12
	J–380	tocopherols	1	143	140	124	75	81	47	16
	G–458		2	126	125	119	88	37	10	20
	G–459		2	184	122	107	81	43	32	21
Group mean				150	131	112	76	49	30	17
III	a									
	H–138	10 g. mixed	1	412	296	234	194	135	87	43
	A–200	tocopherols	7	406	454	373	205	123	49	17
	J–356		2	648	705	520	358	287	158	58
	Subgroup mean			489	485	376	252	182	98	39
	b	0.5–1 g.								
	G–451	tocopherols	2	182	205	87	60	52	21	15
	c	5 g.								
	A–240	tocopherols	2	224	243	150	100	63	39	15
	d	4 g.								
	G–467	tocopherols	1	426	390	266	284	170	124	34
IV	H–126†	None	2	26	28	21	22	20	21	19

* The symbols H, A, J, and G refer to the breeds, Holstein, Ayrshire, Jersey, and Guernsey, respectively; the symbol is followed by the herd number of the cow.

† Cow milked through gestation; no dry-rest period.

for cows on the lower level of supplementation (group II), the initial value was nine times higher, and for cows receiving 10 g. of tocopherols (group III–a), 12 times higher.

Tocopherols in the colostric fat tended to decrease logarithmically during the first 4 days of the transition from colostrum to normal milk (fig. 2).

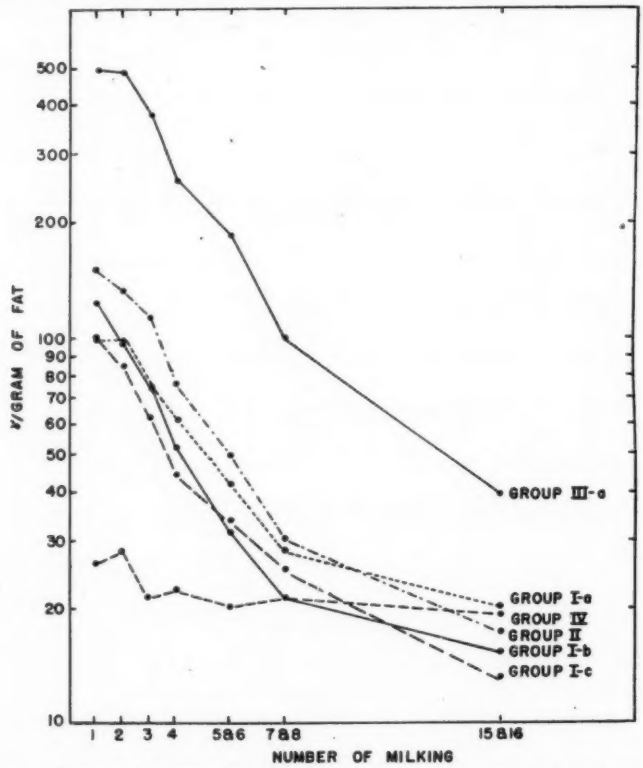

FIG. 2. Changes in concentration of tocopherols in the fat during the transition from colostrum to normal milk (concentration is plotted on a semi-log scale). The first milkings averaged approximately 3 hours postpartum. Group I–a received no vitamin supplements; group I–b received vitamin A alcohol; group I–c received vitamin A ester; group II received vitamin A and 0.5–1 g. of tocopherols; group III–a received 10 g. of tocopherols; group IV received no vitamin supplements and was milked throughout the gestation period.

Subgroup I–a and group III–a included several cows that secreted the highest concentration of tocopherols at the second milking; with these groups the logarithmic decrease commenced after the second milking. The rates of change of tocopherols in the colostric fat were similar for all groups,

the change per gram of fat being between 0.18 and 0.26 γ/milking/γ. In the case of cow H–126 the change was rather erratic, but the general trend suggested a rate of only one-sixth to one-ninth of the foregoing rates. Group III–a, which received 10 g. of tocopherols daily, tended to maintain the initial rate of decline for a longer period than did the other groups. Except for group III–a, after about five to seven milkings a less rapid rate of decline in tocopherol concentration is observed (fig. 2). This condition probably resulted from two factors acting simultaneously: depletion of tocopherol reserves, and continued intake of tocopherols in the standard daily ration consumed during the postpartum period. If a cow were placed upon a tocopherol-free ration at parturition, probably the initial rate of change of tocopherols in the milk fat would be maintained for an extended period, and lower levels would be reached.

As previously indicated, the logarithmic trend seems to be most distinct for individual cows when tocopherols are expressed as concentration per unit of fat rather than as concentration per unit volume of mammary secretion. This suggests that the mammary output of tocopherols depends upon the quantity of fat secreted per unit of time as well as upon the other physiological processes involved in the mobilization and secretion of tocopherols. The indication of a relationship of fat and tocopherols in the mammary secretions is of further interest in light of the evidence, summarized by Hickman and Harris (2), leading to the conclusion that tocopherols, unlike vitamin A, are not associated primarily with fat in plant and animal organisms.

Table 3 shows the total average quantity of tocopherols secreted at the various stages of the colostric period by the cows in each experimental group. The total tocopherol output of the first eight milkings also is given. The range of values again emphasizes the wide individual variations. In general, the differences in the averages for cows of the various groups are similar to those already pointed out in table 2, except the average differences between groups I and II are smaller than when tocopherols are calculated as concentration per unit of fat.

It is of interest to consider the quantity of tocopherols a calf receives during the colostric period. If the calf nurses and, as usual, does not empty the mammary gland, it receives the secretion of lower fat content in a variable and indeterminable quantity. But if it is assumed that a 100-lb. calf consumes daily 10 lb. of a mixed aliquot of a complete milking from its dam, calves from dams receiving no tocopherol supplements (group I) would obtain from 22 to 86 mg. of this vitamin during the first 4 days. If the dams received tocopherols at the 0.5–1 g. level (group II), the calves would obtain 61 to 84 mg. of this vitamin, averaging 30 per cent more than the former group. On the same basis, calves fed colostrum from cows receiving 10 g. of tocopherols daily (group III–a) would consume about five times as

TABLE 3

*Total tocopherols secreted during the colostric period**

Group	Subgroup	Ration supplement		No. of milking						Total first eight milkings
				1	2	3	4	5 & 6	7 & 8	
				Tocopherols, mg./milking						
I	a	None	Av.	38	16	13	19	30	19	135
			Range	20–68	12–21	8–19	14–23	17–39	13–32	92–162
	b	Vit. A alc.	Av.	38	30	26	16	18	14	142
			Range	9–59	10–65	12–38	7–32	8–30	8–23	88–211
	c	Vit. A ester	Av.	39	20	18	11	17	14	119
			Range	8–68	6–32	9–25	4–16	12–23	9–33	79–160
II	–	Vit. A alc. and 0.5–1 g. tocopherols	Av.	47	22	30	24	26	18	167
			Range	4–99	3–52	13–60	12–35	17–38	8–30	89–262
III	a	10 g. mixed tocopherols	Av.	142	93	77	80	135	67	594
			Range	56–220	71–124	45–104	37–104	64–246	29–133	467–833
	b (G–451)	0.5–1 g. tocopherols		81	57	31	15	28	16	228
	c (G–467)	5 g. tocopherols		102	72	36	18	62	29	319
	d (A–240)	4 g. tocopherols		69	80	62	54	74	49	388
IV	(H–126†)	None		6	5	4	6	10	11	42

* First eight milkings.
† Cow milked through gestation; no dry-rest period.

much of these substances as would calves from the unsupplemented dams. The calf of cow H–126, which had no dry-rest period, would have received less than one-half as much tocopherol as a similar calf from a dam subjected to standard herd management, which included a rest period of at least 6 weeks.

Interestingly, by the fourth day cow H–126 secreted into 10 lb. of milk practically as large a quantity of tocopherols as the average of all experimental animals not supplemented with tocopherols (group I) and 60 per cent of the quantity secreted by cows receiving these substances at the 0.5–1 g. level (group II). In contrast, cows receiving 10 g. of mixed tocopherols (group III–a) supplied an average of four times more of this vitamin during the fourth day than did cow H–126.

At birth the tocopherol content of the blood serum of the calf was low but increased markedly upon ingestion of colostrum, the magnitude of the response varying with the tocopherol content of the colostric secretion (3). The response of the calf substantiates the direct measurements reported herein and is in accord with rat investigations (4, 5).

SUMMARY

Tocopherol levels were determined in the mammary secretions of dairy cattle during the period of transformation from colostrum to normal milk. The limited data revealed no differences that could be attributed either to the breed or to the number of previous lactations.

For cows receiving tocopherols at 0, 0.5–1 g., and 10 g. levels of supplementation during the latter stages of gestation, the concentrations of these substances in the first colostrum were in the ratio of 1, 1.4, and 4.5, respectively. The average levels of tocopherols in the first colostrum from the same three groups were, respectively, 7, 9, and 12 times higher than in the milk on the eighth day.

The change in tocopherol concentration in colostric fat followed a logarithmic trend that was similar for each of the experimental groups. Cows on the higher plane of supplementation tended to maintain the initial rate of change over a longer period of time than did those on a lower level of tocopherol intake.

For the cow that was milked through the gestation period, tocopherol levels of the first postpartum secretions were much lower and the rate of decline was less than for cows having the normal dry-rest period. However, values for this cow were practically as high by the end of the colostric period (4 days) as were those of cows not receiving supplements of tocopherols but allowed the dry-rest period.

Calculations were made showing the quantities of tocopherol that a calf would receive during the colostric period through consuming colostrum from cows on the various levels of supplementation.

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PARTURIENT PARESIS. I. THE INCIDENCE OF PARTURIENT PARESIS AND CHANGES IN THE TOTAL BLOOD SERUM CALCIUM AT PARTURITION IN PREPARTUM MILKED COWS[1]

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Dryerre and Greig (2) postulated that the marked decline in blood serum calcium at parturition was the result of the sudden initiation of lactation. Turner (7) suggested that prepartum milking may reduce the incidence of parturient paresis or milk fever by a gradual initiation of lactation. In order to provide more conclusive information on this subject, an experiment was designed to ascertain the effects of prepartum milking on incidence of parturient paresis and upon the level of total blood serum calcium.

EXPERIMENTAL METHODS

This experiment was conducted on the Jersey herd of Biltmore Farms, Biltmore, North Carolina. At present the herd consists of approximately 600 milking cows grouped into ten functional units. A maternity barn is maintained for calving cows for three of the units. With the exception of first-calf heifers, practically all of the cows that calved at the maternity barn for 7 consecutive weeks (February 28 to April 17, 1947, inclusive) were prepartum milked. Eleven cows calving at outlying units during the same period also were included.

An effort was made to milk all cows 10 days prepartum, based on the expected calving date and depending on when the cow was placed in maternity quarters. Usually prepartum milking began before the udder showed evidence of congestion. The cows were hand-milked twice daily. Milk production weights were taken for each prepartum milking. Calves were left with their dams from 1 to 3 days.

Blood samples were drawn daily from 3 to 5 days before the anticipated day of parturition, and usually 3 days subsequent thereto. Ordinarily samples were drawn between 8:00 and 9:00 a.m. In the event of parturient paresis, a blood sample was drawn immediately before treatment. Cows were not treated until definite comatose symptoms were evident. The blood serum samples were shipped in refrigerated cartons three times weekly to Madison, Wisconsin, for analysis. Total serum calcium was determined by the method of Clark and Collip (1).

A summary was made of the total number of calvings and the incidence

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TABLE 1

Changes in the total blood serum calcium at parturition in prepartum milked cows

Cow no.	No. of parturition	Days milked prepartum	Lb. milk produced day previous to parturition	Total blood serum calcium (mg.%)						
				Days prepartum			Day of parturition	Days postpartum		
				3	2	1		1	2	3
16	3	11	24.7	10.3	9.7	9.7	8.9	8.2	9.4	10.0
38	2	14	25.1	9.5	9.3	9.6	9.4	9.3	10.1	10.5
47	2	3	8.5	*	*	*	6.6	6.6	8.9	10.1
50	2	6	10.5	*	9.0	9.0	8.6	8.3	10.1	10.2
56	2	6	12.5	10.7	10.6	9.8	9.3	8.5	9.4	10.6
112†	8	12	17.6	8.9	9.5	9.0	6.6–5.0‡	9.9	8.7	10.1
113	2	4	7.3	*	*	*	8.8	8.9	11.1	10.6
129†	6	12	8.0	11.1	10.7	*	3.9‡	3.7	8.8	9.7
130	2	8	20.2	10.8	10.3	9.0	8.7	9.0	10.6	10.2
132	10	8	16.0	7.3	6.5	9.0	10.0	7.8	9.6	*
151	2	9	23.5	10.4	10.4	9.4	9.2	9.8	10.1	10.2
163	6	10	17.0	9.4	10.9	12.5	8.7	9.2	9.5	9.9
168	8	5	11.7	9.8	8.7	9.5	7.7	7.6	8.7	9.3
203	2	10	6.0	10.4	8.9	9.9	9.0	8.6	8.3	10.7
205	2	14	§	10.2	10.8	9.9	9.4	10.2	10.2	10.7
225	2	5	1.3	9.9	10.2	9.7	*	7.7	9.2	9.1
236	2	11	14.2	*	10.7	12.2	9.7	8.6	9.1	*
249	2	5	0.9	10.5	11.2	10.5	9.8	9.3	10.1	10.0
267	5	4	0.4	*	10.4	10.9	10.7	10.2	9.2	7.7
284	6	6	1.4	10.2	10.4	10.7	8.3	5.8	9.2	10.1
321†	5	10	3.1	10.8	10.6	11.0	6.1–3.8‡	5.4–4.0‡	7.8	9.2
354	5	13	24.1	9.9	9.5	8.9	7.1	7.6	10.0	10.1
367	5	13	6.5	*	*	*	6.5	7.9	9.2	9.4
372†	5	11	11.0	11.1	11.0	10.1	6.5–4.5‡	*	8.0	8.8
410	5	11	23.0	10.0	10.0	11.0	8.7	8.1	*	10.5

TABLE 1—(*Continued*)

Cow no.	No. of parturition	Days milked prepartum	Lb. milk produced day previous to parturition	Total blood serum calcium (mg.%)						
				Days prepartum			Day of parturition	Days postpartum		
				3	2	1		1	2	3
435	5	3	§	*	9.8	9.5	8.3	9.2	10.1	10.5
445	4	7	15.4	*	*	8.8	8.4	8.8	8.7	9.6
447	4	16	27.0	9.6	10.0	9.7	8.9	9.1	8.9	9.7
450	4	13	8.7	10.6	12.4	11.5	7.8	8.5	*	*
464†	5	8	5.2	*	*	9.4	6.1–4.0‡	6.1	5.7	*
512†	4	11	12.7	*	10.0	11.4	8.7–5.9‡	8.4–5.9‡	6.7	10.5
520	4	6	9.1	10.7	9.4	9.5	7.4	8.4	9.5	*
541	4	7	31.0	7.2	9.0	8.6	7.7	9.7	10.0	*
575†	6	9	1.1	10.9	*	10.1	6.9–3.9‡	5.9	*	8.5
577	3	2	6.2	11.8	10.3	9.7	9.1	10.2	11.7	10.4
580	4	2	§	*	10.0	9.5	8.6	9.6	10.3	10.6
583	3	14	32.2	9.8	9.6	9.4	8.0	8.5	10.4	*
588	3	6	15.5	11.8	10.2	9.3	9.8	8.9	9.4	10.3
590	4	3	§	9.2	10.2	10.2	9.6	9.0	10.5	11.1
595	3	15	23.4	*	8.9	9.5	9.4	8.6	10.3	10.4
597	3	2	0.3	*	*	*	8.6	7.2	9.7	9.6
M30†	4	9	0.5	11.1	11.4	9.2	5.5	5.1–4.9‡	8.0	*
M41	4	15	6.3	11.1	10.1	9.3	6.4	7.6	*	*
M82	8	8	11.5	10.9	10.7	10.4	7.8	6.5	8.9	*
M104†	6	7	16.2	*	*	*	*	*	*	*
R	?	6	2.0	11.0	11.6	11.3	8.7	10.0	*	*

* Either blood sample was not drawn, or the sample accidentally destroyed.
† Had parturient paresis.
‡ Blood sample taken before treatment for parturient paresis.
§ Milk not weighed.

of parturient paresis of cows calving at the maternity barn for the 7 weeks prior to and subsequent to the period when the cows were prepartum milked.

An analysis was made of the health records of cows calving at the maternity barn for the years 1943 through 1946, inclusive, excluding first-calf heifers. The herd was under the supervision of a veterinarian during the entire period, and complete records of all treatments were kept by the attendant at the maternity barn.

For the purposes of this study a "normal calving" will indicate there were no symptoms of parturient paresis.

RESULTS

The results of the study are presented in tables 1, 2, and 3. Forty-six cows were prepartum milked for 2 to 16 days. On the day previous to parturition, milk production ranged from 0.3 to 32.2 lb., with an average of 13.0 lb. Nine of the forty-six, or 19.6 per cent of the cows, had parturient paresis. Average milk production the day previous to parturition for those

TABLE 2

The effect of prepartum milking on the incidence of parturient paresis

	Period of:		
	Control	Prepartum milking	Control
	Jan. 10–Feb. 27	Feb. 28–Apr. 17	Apr. 18–June 5
Total parturitions	45	46	33
Parturient paresis	11	9	11
%	24.4	19.6	33.3

cows having parturient paresis was 8.4 lb., and for cows with normal parturitions, 13.4 lb.

The incidences of parturient paresis during the 49-day periods before and following the period of prepartum milking are presented in table 2. Of the 45 cows that calved in the 49-day period just preceding that in which prepartum milking was done, 11 or 24.4 per cent had parturient paresis. Thirty-three cows calved in the first 49 days immediately following the period of prepartum milking, of which 11 or 33.3 per cent had parturient paresis. The difference in the incidence of parturient paresis during the prepartum milking period and control periods is not statistically significant by the chi-square test of independence and association. The incidence of parturient paresis in the same herd for the 4 previous years was 19.3 per cent (table 3).

Cow 112 appeared lethargic the morning of the day of parturition, and her condition grew progressively worse toward evening, at which time she was staggering. The attending veterinarian diagnosed her condition as

parturient paresis. Subsequent analysis showed the total serum calcium level to be 5 mg. per cent. A posterior pituitary extract was given, and delivery of the calf was manually aided.

Cow M104 was housed at one of the outlying units and blood samples were not obtained. This cow was unable to arise the day after parturition, and her condition was diagnosed by the veterinarian in charge as parturient paresis. She responded to calcium gluconate therapy.

Thirty-five of the forty-four cows on which total serum calcium was determined showed a definite drop in the calcium level the day of or day after parturition. Three other cows, 47, 113 and 367, had relatively low total

TABLE 3

Incidence of parturient paresis of cows calving at maternity barn of Biltmore Farms (1943 through 1946, inclusive, excluding first-calf heifers)

Months	Total no. of parturitions	Parturient paresis	Parturient paresis
			(%)
Jan.	111	26	23.4
Feb.	130	38	29.2
March	123	13	10.6
April	105	27	25.7
May	105	27	25.7
June	97	15	15.5
July	111	23	20.7
Aug.	108	22	20.4
Sept.	63	8	12.7
Oct.	72	7	9.7
Nov.	81	10	12.3
Dec.	77	12	15.6
Total	1183	228	19.3
May–Sept.	484	95	19.6
Oct.–April	699	133	19.0

serum calcium levels on the day of parturition, but previous blood samples were not obtained, so no comparison could be made. Cows 132 and 541 had relatively low total serum calcium levels before parturition.

DISCUSSION

A comparison of tables 1, 2, and 3 shows that prepartum milking as performed in this study did not reduce the incidence of milk fever. In some cows, milk secretion was not stimulated by prepartum milking, even though milking began several days before parturition. Although average milk production was not as high in cows having parturient paresis, milk flow was well established in five of these cows—112, 129, 372, 512, and M104. With cow 112, parturient paresis occurred before parturition. Preparturient paresis is not uncommon, as statistics compiled by Jensen (5) showed that parturient paresis occurred before parturition in 4.24 per cent of 1,107 cases.

There were no marked differences between the summer and winter months

on the incidence of parturient paresis in this herd (table 3). Generally, these cows are turned to pasture the first 2 weeks of March. Parturient paresis occurred just as frequently in the months from May through September as from October through April. This observation is in agreement with Hibbs *et al.* (4), but at variance with Metzger and Morrison (6) and Henderson (3).

SUMMARY

Forty-six cows were prepartum milked from 2 to 16 days. Nine of these cows had parturient paresis.

Prepartum milking did not significantly reduce the incidence of parturient paresis.

It would seem from the results of this study that factors in addition to the initiation of milk secretion are involved in causing parturient paresis.

The incidence of parturient paresis is not seasonal in this herd.

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THE EFFECT OF FEEDING CERTAIN SYNTHETIC VITAMINS OF THE B COMPLEX ON THEIR CONTENT IN MILK AND URINE

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It is generally believed that the B vitamins are not essential in the diet of mature ruminants for normal growth, lactation, and reproduction. Wegner *et al.* (18) have demonstrated the ability of the bovine to synthesize a significant amount of thiamin, riboflavin, pyridoxine, biotin, nicotinic acid, and pantothenic acid when fed a ration very low in these factors. McElroy and Goss (9, 10) indicate that thiamin is not a dietary essential for ruminants, but that the cow and the sheep synthesize this vitamin in the rumen and reticulum. The work of these investigators indicated the synthesis of thiamin, riboflavin, pyridoxine, and pantothenic acid in the ruminant. These vitamins were reported as being formed either in the body tissue or by microorganisms in the rumen. Nicotinic acid also was reported as being synthesized in the ruminant by Winegar *et al.* (20). Bechdel *et al.* (1) found the rumen microflora to be 90 per cent flavobacterium (their own designation). This organism was fed to rats after having been grown in a B complex-free media and found to be highly potent in the vitamin B complex. This probably explains why cattle can produce normal offspring and milk on a ration that is deficient in the vitamin B complex for rats.

Williams *et al.* (19) determined eight vitamins of the B complex in milk from different species. They reported the vitamin levels in the different milks generally to increase progressively from human beings and the mare to the mouse. This increase in the content of these vitamins in milk of the different species is analogous with the rate at which these species reach maturity. This would seem to indicate that the B complex is a factor in growth rates. Pearson and Darnell (12) showed that colostrum from either the cow or the ewe was considerably higher in thiamin and riboflavin than was the normal milk of the respective species. Since nature provides an abundant supply of these two vitamins, possibly they are essential for early development and growth of young mammalia. There also are seasonal variations in thiamin and riboflavin, as well as ascorbic acid, as shown by Holmes *et al.* (4). The change from a dry roughage to silage during the months of December and January caused a sharp increase (36 per cent within 2 days) in riboflavin content in Jersey and Holstein milk as shown by Theophilus and Stamberg (17). Considerable work has been done on feeding diets low in B vitamin, synthesis by the ruminant, and the effect of ration on B vitamin content in milk. A survey of the literature reveals no work concerning

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the feeding of these vitamins in the synthetic form and their effect on the contents in the milk. Since milk constitutes an important item in our diet, experimental work was undertaken to study this problem in dairy animals.

EXPERIMENTAL

Work was started with milking cows. Goats were used later to reduce the amount of synthetic vitamins fed and to permit the use of a metabolism cage for the collection of urine. A purebred Jersey cow and a purebred Holstein cow were selected from the college dairy herd. Since according to Pearson and Darnell (12) the amount of certain B complex vitamins is higher in early lactation, cows and goats were selected that were far enough along in lactation to assure normal milk. Previous lactations of these animals were checked and found to be normal.

The goats used in this experiment were reported by their previous owners to be purebreds—one a Saanen and the other a Toggenburg. They were stall-fed without access to pasture throughout the experiment. The concentrate ration for each species was the ration used for the milking herd. A fair quality alfalfa hay was used, along with sorghum silage; however, the cows had access to pasture and as the experiment ran through the spring flush season, some changes in the intake levels of some of these vitamins may have occurred [Holmes *et al.* (4)].

Preliminary studies were completed on April 11, 1946, and the cows were placed on rations that had been supplemented with 500 mg. each of synthetic thiamin, riboflavin, nicotinic acid, and pantothenic acid. All four of these vitamins were fed concurrently to the cows as well as the goats. Daily collection of samples began with the first milking on April 12, 1946. Collections were made at this level for a period of 6 consecutive days; then the supplement was increased to 2 g. daily and collections were taken for 10 consecutive days at this level. A further study was conducted at a 16-g. level. During this period one cow was fed 16 g. of each of the vitamins for a period of 3 days. Samples of milk were taken on the second and third days and assays run on the samples.

Goat no. 1, a Toggenburg, was placed in the metabolism cage for a period of 2 days on May 28, 1946, and a preliminary study was made while the goat was on the basal ration. In this case samples of milk, urine, and blood were taken and vitamin determinations were run on each of these products with the exception that thiamin was not determined in blood. On June 7, goat no. 1 was placed in the metabolism cage for a period of 10 days and given the basal ration plus 2 g. of each of the vitamins for the first 5 days, no supplement being given during the last 5 days. This gave a study period of 12 days during which time daily collections of milk and urine were made. The blood samples were taken on the second, seventh, and twelfth days during the study period. Goat no. 2, a Saanen, was given treatment identical

to that of goat no. 1. Further study on each goat was made at a low level of 250 mg., which is approximately equivalent to the 2-g. level for the cows, based on body weights of the two species. During this period of 4 days the goats were fed 250 mg. of each of the vitamins, and composite samples were made from daily collections of milk and urine. The blood samples were taken on the third day.

The cows and goats were milked twice daily. Samples were taken from a.m. and p.m. milkings and a composite made for that day. The urine was collected from the goats by means of the metabolism cage and blood samples taken from the jugular vein. To prevent photochemical destruction of riboflavin (15), all samples of milk and urine were collected in brown bottles and stored in the dark at a temperature of 0 to 4° C. All laboratory work with this particular vitamin was carried out under subdued light.

Thiamin was determined by the fluorometric method of Hodson (3). Riboflavin was determined by the microbiological method of Snell and Strong (14), with a slight modification devised by Isbell (6). Nicotinic acid was determined by the microbiological method of Krehl *et al.* (8), with a slight modification devised by Isbell (6). Pantothenic acid was determined by the microbiological method of Strong *et al.* (16), as modified by Neal and Strong (11).

RESULTS

The data on the vitamin content of the milk from the cows are summarized in table 1. The average thiamin content of the milk of the Jersey and Holstein while on the basal ration was 44 μg. and 34 μg. per 100 ml. for the respective animals. These values compare favorably with the values ranging from 41 to 53 μg. per 100 ml. for milk from a mixed herd of dairy cows (5). The thiamin values during the period of supplementation with 500 or 2,000 mg. daily of thiamin were not significantly different from the values on

TABLE 1

The effect of the ingestion of thiamin, riboflavin, nicotinic acid, and pantothenic acid on the levels in the milk
(Values in μg. per 100 ml.)*

Vitamin supplement	Jersey			Holstein		
	Values on basal ration	Levels of vitamin ingested		Values on basal ration	Levels of vitamin ingested	
		500 mg.	2000 mg.		500 mg.	2000 mg.
Thiamin	44	38	42	34	36	39
Riboflavin	178	198	167	98	103	88
Nicotinic acid	104	123	119	82	90	89
Pantothenic acid	341	329	399	275	268	309

* Values on basal ration average for 2 days, values during periods of vitamin supplementation average for 6 days.

the basal ration, indicating that the feeding of synthetic thiamin is not effective in increasing the concentration in the milk. One cow was force-fed 16 g. of thiamin without any significant increase in the concentration of this vitamin in the milk. The milk of the goats fed 250 mg. of thiamin did not show any increase in thiamin level over the period during which the basal ration was fed. When 2 g. of thiamin were fed daily to goats, there was a significant increase in the level in the milk and a very marked increase in the amount excreted in the urine (fig. 1).

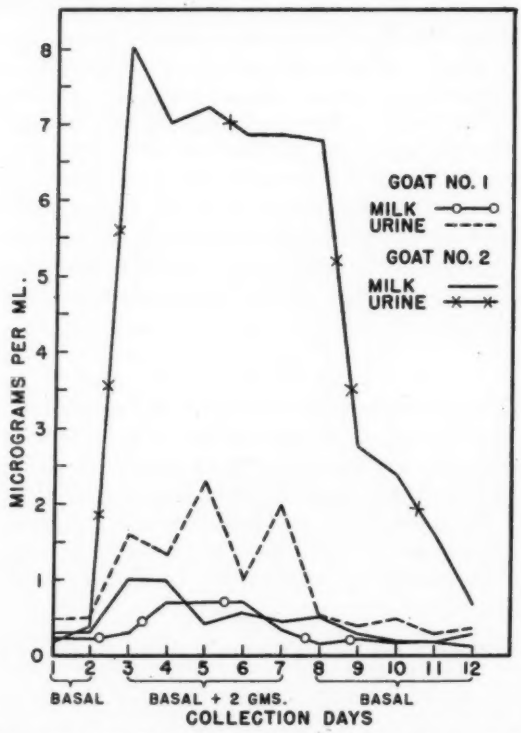

FIG. 1. The effect of the daily ingestion of 2 g. of thiamin by goats on the levels of thiamin in the milk and urine.

The feeding of synthetic riboflavin, nicotinic acid, and pantothenic acid to cows at levels of either 500 or 2,000 mg. daily for periods of 6 days did not increase significantly the concentration of these vitamins in the milk. The feeding of a massive dose of 16 g. of each of these three vitamins was without appreciable effect in increasing the concentration of these vitamins in the milk. The milk of the cow fed 16 g. of riboflavin had a distinct green-

ish-yellow color characteristic of aqueous solutions of riboflavin. Milk from the cow and goats fed the higher levels of riboflavin gave a much higher value for riboflavin when determined fluorometrically than when measured microbiologically. An account of this phase of the work is reported in a separate paper (13).

The daily feeding of 250 mg. of riboflavin, nicotinic acid, and pantothenic acid did not result in a significant increase in the concentration of any of the vitamins in the milk. The feeding of 2 g. daily of each of these vitamins

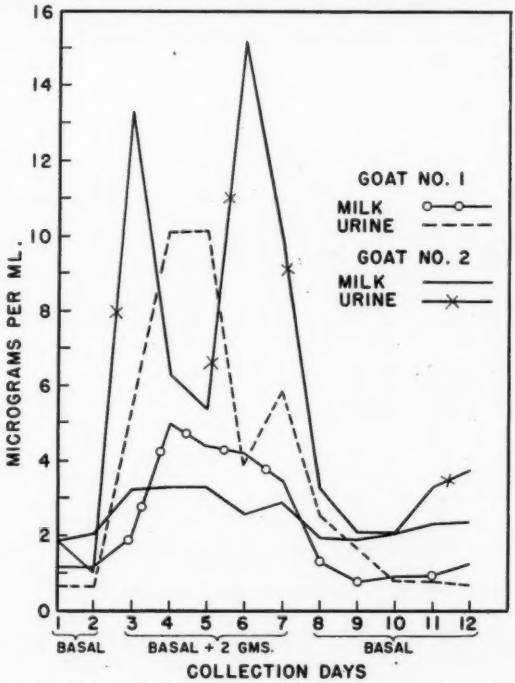

FIG. 2. The effect of the daily ingestion of 2 g. of riboflavin by goats on the levels of riboflavin in the milk and urine.

to goats resulted in a significant increase in the concentration of each in the milk and a very marked increase in the amount in the urine (figs. 2, 3, and 4).

DISCUSSION

The possible destruction of the water-soluble vitamins, as in the case of ascorbic acid, must be kept in mind [Knight *et al.*(7)]. There appeared to be no significant increase in content in cow's milk of any of the four vitamins

studied at the level fed in these studies. An analysis of variance of the data was conducted to determine breed differences in milk levels relative to the four vitamins studied. In each case there was found to be a significant breed difference on a unit basis. The average values observed were: thiamin, 44 μg. per 100 ml. in Jersey milk as compared to 34 μg. per 100 ml. in Holstein milk; riboflavin, 198 μg. per 100 ml. in Jersey milk and 98 μg. per ml. in Holstein milk; nicotinic acid, 104 μg. per 100 ml. in Jersey milk and 82 μg. per ml. in Holstein milk; and pantothenic acid, 341 μg. per 100 ml. in Jersey milk and 275 μg. per 100 ml. in Holstein milk. These values were for normal

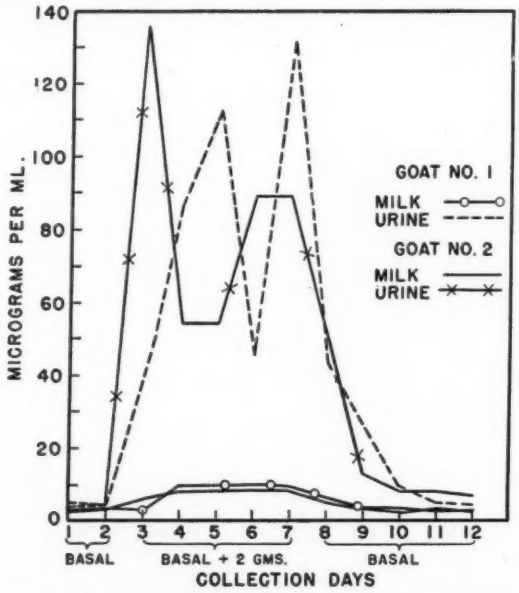

FIG. 3. The effect of the daily ingestion of 2 g. of nicotinic acid by goats on the levels of nicotinic acid in the milk and urine.

milk while the cows were on the basal ration. As shown, the Holstein ran consistently lower than the Jersey on a milliliter basis; however, on total daily milk production, the Holstein cow had a much greater output than the Jersey. This agrees with similar work with riboflavin by Theophilus and Stamberg (17).

In comparing the vitamin content in milk of the two species, the following observations were made on a unit basis and by using averages of the two breeds of each species. For thiamin the goats were found to have 29 μg. per 100 ml. compared to 36 μg. per 100 ml. for the cows. This figure is contradictory to a report by Gamble *et al.* (2), in which they state that Toggen-

burg and Saanen milk contained 1 I.U. of thiamin in 3 to 4 ml. as compared to 1 I.U. of thiamin in 5 to 7 ml. of Holstein and Jersey milk. This is in agreement with work by Williams *et al.* (19). Riboflavin content in the goat milk was 157 μg. per 100 ml. as compared to 138 μg. per 100 ml. for the cows, which is in agreement with work by Gamble *et al.* (2) based on rat feeding assays. The values for the goats are slightly higher than the value given by Williams *et al.* (19), which was 110 μg. per 100 ml. The nicotinic acid content for goat milk was found to be almost three times as high as for cow's milk, the values being 267 μg. per 100 ml. for goats and 93 for cows. Pearson and Darnell (12) reported the average nicotinic acid content for cow's

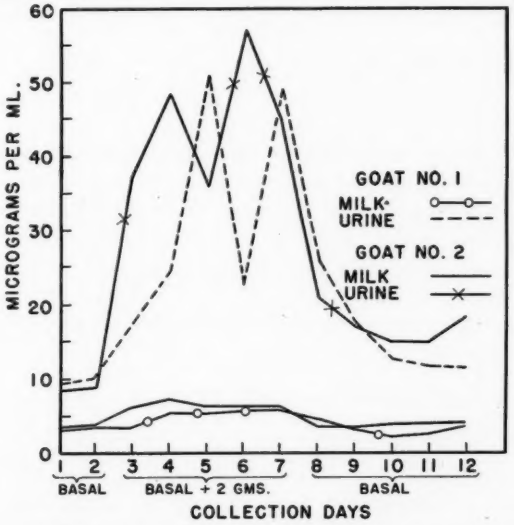

FIG. 4. The effect of the daily ingestion of 2 g. of pantothenic acid by goats on the levels of pantothenic acid in the milk and urine.

milk as 91 μg. per 100 ml., and Williams *et al.* (19) reported goat's milk as having 250 μg. per 100 ml. This work agrees with those reports and certainly indicates a very distinct species difference in levels of nicotinic acid in the milk. The pantothenic acid content was found to be 322 μg. per 100 ml. in goat's milk compared to 306 μg. per 100 ml. in the cow's milk. This work indicates no species difference in pantothenic acid content of milk and agrees with work of Pearson and Darnell (12) in which they report 367 μg. per 100 ml. in cow's milk. Williams *et al.* (19) report the value for goat's milk as 240 μg. per 100 ml., which is only slightly lower than values reported in the present study.

Assays were made on the blood for riboflavin, nicotinic acid, and pantothenic acid by the microbiological procedures. Blood samples were collected

from the goats before, during, and after the feeding of the vitamins at the 2-g. level. The blood values for riboflavin ranged from 21 to 36 μg. per 100 ml. There was no consistent difference in the riboflavin content of the blood of the goats during the period they were on the basal ration and the period they were receiving riboflavin. The ingestion of the nicotinic acid increased the level on the basal ration from 560 to 624 μg. to a maximum of 948 and 1,034 μg. per 100 ml. of blood for the respective goats. The pantothenic acid values on the basal ration were 78 and 65 μg. per 100 ml. of blood. During the period of pantothenic acid feeding, the maximum values for pantothenic acid were 140 and 184 μg. per 100 ml. of blood.

SUMMARY

1. Synthetic thiamin, riboflavin, nicotinic acid, and pantothenic acid fed at a 2-g. level daily to dairy cows resulted in no significant increase in the levels in the milk.

2. There appeared to be a distinct breed difference in the cows in amount of these B vitamins in the milk on a unit basis, the Jersey being higher than the Holstein in micrograms per milliliter, while the Holstein was higher in total amount secreted daily, based on total milk production.

3. In the case of the goats a significant increase of each of the vitamins occurred after they had been fed at the 2-g. level; partial destruction was indicated, since the per cent recovered in the milk and urine was 10 per cent or less of the amount ingested.

Acknowledgment is made to Merck & Co. for the synthetic vitamins used in these investigations.

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STUDIES ON MILK PROTEINS. I. CONDITIONING OF MILK PROTEINS WITH PANCREATIC ENZYMES

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When milk is treated with minute amounts of active pancreatic enzymes and pasteurized immediately, its proteins undergo modifications. This change is evidenced by the fact that hard, strong clots no longer can be formed through the use of acids or coagulating enzymes such as pepsin or rennin. Conquest *et al.* (1) have described this action in connection with milk of reduced curd tension. Casein primarily is affected according to the work of Turner (3), who has obtained a greater solubility in 70 per cent ethyl alcohol in this protein separated from pancreatic enzyme-treated milk than that found in a normal control.

The laboratories of Armour and Company have been interested in studying caseins precipitated from acidified, pancreatic enzyme-treated milks and have noted a change somewhat different from the above. These caseins appear to be affected by heat in a manner opposite to the untreated control. For example, temperatures in excess of 150° F. will cause the enzyme-treated casein to melt and flow, whereas untreated caseins become stringy, then hard, and finally glassy. Previous investigations reported by Conquest *et al.* (1), Doan and Flora (2), and Turner (3) have disclosed small chemical differences between pancreatic enzyme-treated milk and the untreated milk. Since these chemical changes seem diminutive when compared to the physical ones noted in the following experiments, other factors may be involved. In this connection one could predict molecular dissociation wherein a limited number of chemical bonds are ruptured. For purposes of studying this phase, the principles of osmosis have been utilized. Such technique, properly controlled, should reveal whether appreciable molecular dissociation or cleavage had transpired.

EXPERIMENTAL PROCEDURE

A cellophane tube listed as Sargent S–25275, 28 mm., was used. Sacs were formed from it by cutting 8-inch lengths and tying one end with a string. One-hole rubber stoppers were held in the tops of the cellophane sacs by glass bands cut from large tubes and slipped up over the sacs to give strong joints. Calibrated glass tubing of 7 mm. size was inserted in the stoppers for containing the liquid increase and for providing a means for its measurement.

The milk to be tested first was warmed to 25° C., and 70-ml. portions were measured into the cellophane sacs. The sacs were immersed at equal

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depths in running distilled water at 25° C. Readings of liquid column heights were made hourly. Determinations always were run parallel in order better to eliminate discrepancies due to variations in barometric pressure. Experimental portions were removed from a large lot of raw milk so that uniformity might prevail. A commercial preparation of pancreatic enzymes used for producing soft curd milk, and sold under the trade name

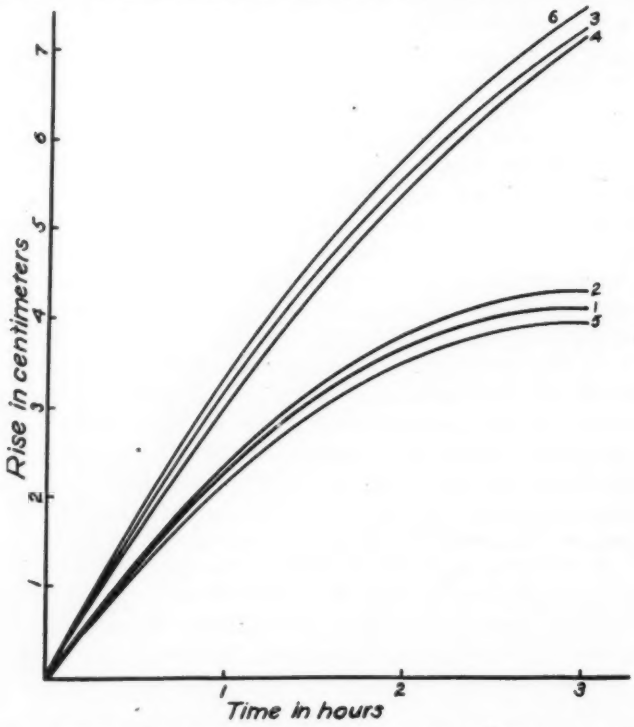

FIG. 1. Osmotic rates obtained on experimental milks at 25° C. 1. "Enzylac" enzyme added to the cold milk and then pasteurized. 2. Inactivated Enzylac enzyme added to the cold milk and then pasteurized. 3. Enzylac enzyme added to the cold milk, then pasteurized, when Enzylac enzyme again was added. 4. Milk first pasteurized and then Enzylac enzyme added. 5. Inactivated Enzylac enzyme added. 6. Enzylac enzyme added to milk; no subsequent treatment.

of "Enzylac," was the enzyme source. Whenever used, it was added at the recommended rate of 1:10,000 or 0.01 per cent to the cold, raw milk. Pasteurization was started immediately and a temperature of 145° F. was maintained for 30 minutes according to standard procedure.

All digestive enzymes of animal origin are inactivated by heating in

the range of pasteurization, so this step terminated further action of the Enzylac on milk proteins. Experimental milks employed in this study were made from one lot of raw milk.

The curves (fig. 1) represent typical osmotic rises, obtained on these milks, plotted against time, thereby showing osmotic rate.

RESULTS

Curve 1 shows Enzylac-treated milk with a curd tension of 25 to give the same rate of osmosis as milk which was pasteurized and had a curd tension of 60 (curve 2). Raw milk (curve 5) essentially is in a similar range with the other two samples.

Curves 3, 4, and 6 demonstrate the osmotic rate observed when digestion by active pancreatic enzymes is in progress. Obviously the increased rate of these milks goes far beyond the other samples.

From these osmotic studies it is apparent that milk treated with pancreatic enzymes and pasteurized is comparable to either pasteurized or raw milk with respect to numbers of particles contained in equal volumes. Thus, chemical differences between enzyme-treated milk proteins and controls would seem to be due to small disintegrations at the molecular surface rather than deep cleavages.

SUMMARY AND CONCLUSIONS

Apparently the pancreatic enzyme treatment recommended for making a milk of low curd tension induces a weakening of the internal structure of casein. In this condition the molecules are rendered susceptible to dissociation by heat. For descriptive purposes, this unstabilizing action is referred to as "conditioning".

Physical measurements rather than chemical methods seem to have greater possibilities for measuring this effect.

Work on both physical and chemical methods for determining degree of "conditioning" or change in molecular structure is being continued.

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STUDIES ON MILK PROTEINS. II. COLORIMETRIC DETERMINATION OF THE PARTIAL HYDROLYSIS OF THE PROTEINS IN MILK

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The need to detect and measure smaller degrees of protein hydrolysis than can be detected by available methods occurs frequently enough in the laboratory to warrant research on methods.

One problem under study involves the treatment of liquid milk with small amounts of pancreatin for the reduction of curd tension. The degree of breakdown of the protein structure cannot be determined by conventional methods, such as the Sorensen formol titration. Turner (4) reported a greater alcohol-soluble fraction from trypsin-treated milk. However, this method does not give results uniform enough to be translated into the degree of protein hydrolysis. Some methods require special equipment and techniques, whereas others use purified substrates that make them less adaptable to a complex mixture containing protein such as milk.

The method of Anson (1), using a denatured hemoglobin solution as a substrate to measure proteolytic activity of enzymes, was found to be the most adaptable for measuring the hydrolysis of milk protein. The enzymes liberate enough of the amino acids tyrosine and tryptophane from the hemoglobin so that when the phenol reagent of Folin and Ciocalteau (2) is added to a sample of the protein-free filtrate, a blue color is formed which can be measured colorimetrically.

EXPERIMENTAL PROCEDURES

1. *Phenol reagent.* To the reagent prepared according to Folin and Ciocalteau (2), two volumes of water are added. This is the same reagent that Kay and Graham use in their phosphatase test (3).

2. *Trichloroacetic acid.* A 0.72 N solution is prepared from reagent grade trichloroacetic acid.

3. *Sodium carbonate–sodium tetraphosphate solution.* Seventy-five grams of anhydrous sodium carbonate and 10 g. of sodium tetraphosphate (Quadrafos) are dissolved in water and diluted to 500 ml.

4. *Tyrosine standard solution.* Eastman tyrosine recrystallized and dried is used. A nitrogen determination should be run to determine the purity of the product. One hundred milligrams of the tyrosine are dissolved in water and enough formaldehyde is added to prevent bacterial growth; the solution then is diluted to 500 ml.

Methods. When milk is substituted for the denatured hemoglobin solu-

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tion, several changes have to be made in the reagents. The method of Anson (1) calls for a 0.3 N trichloroacetic acid solution to precipitate the protein, hemoglobin. The concentration must be increased to 0.72 N in order to remove the milk proteins.

In preparing the protein-free filtrate for addition of the phenol reagent, it is advisable to use a 15 per cent sodium carbonate solution containing enough polyphosphate to prevent the precipitation of the calcium salts of milk during the addition of the carbonate.

Since milk and enzyme preparations normally contain some color producing products, as shown by this test, blanks must be run on them in order to correct for this color formation.

A 5-ml. quantity of milk is pipetted into a clean dry test tube; 1 ml. of distilled water is added, followed by 10 ml. of 0.72 N trichloroacetic acid while agitating the test tube to mix the milk. The tube then is stoppered, shaken vigorously, and allowed to stand for 10 minutes before filtering the contents through an S&S no. 595 filter paper. Five milliliters of filtrate are added to a 50-ml. Erlenmeyer flask and 10 ml. of the sodium carbonate reagent added and mixed thoroughly before 3 ml. of phenol reagent are added. The sample is shaken continuously while adding the phenol reagent. About 5 minutes are allowed for the blue color to reach a maximum before any readings are made.

The blue color is measured in a Coleman spectrophotometer, using 650 mμ wave-length light. The color also can be measured satisfactorily by using a photometer with appropriate glass filters that transmit light in the 650 mμ wave-length range. A previously prepared standard tyrosine curve showing the per cent light transmission for various tyrosine concentrations then is used to convert the sample reading into its tyrosine equivalent.

Milk protein hydrolysis is demonstrated very readily by the addition of various amounts of pancreatic enzymes to the milk and allowing the enzyme to react for short periods. Five milliliters of milk are pipetted into the test tubes and the required amount of water added to make up the difference between the volume of the enzyme solution added and 1 ml. After bringing these test-tube samples to the desired temperature in a water bath, the required amount of enzyme solution is added. At the end of a 10-minute period the enzyme action is stopped by adding 10 ml. of trichloroacetic acid. A blank on the enzyme solution is prepared by adding the required amount of this solution to the substrate after the trichloroacetic acid has been added to the milk.

The standard tyrosine curve was prepared by adding various amounts of tyrosine to a series of test tubes, and making up to a total volume of 6 ml., followed by the trichloroacetic acid. The remainder of the procedure was the same as that used for a milk sample except that filtering was not necessary.

The concentration of tyrosine in the final 5-ml. aliquot used for producing the color may be up to 0.15 mg. without producing too much color to be measured accurately. By plotting the per cent light transmission against the tyrosine concentration (in the final 5-ml. aliquot) on semi-log graph paper, a straight line was produced.

RESULTS

The results obtained for producing the standard tyrosine curve are given in figure 1.

Some typical results of the action of a sample of Armour's Pancreatin U.S.P. upon milk are given in figure 2.

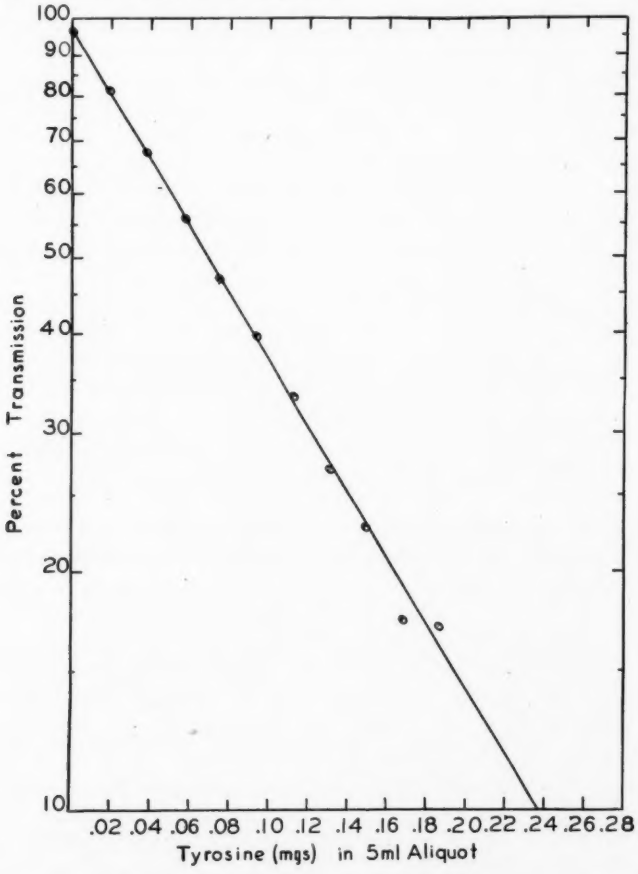

FIG. 1. Spectrophotometer measurement of color produced from tyrosine and phenol reagent at 650 mμ.

In calculating the results of this test, the tyrosine content is based upon its concentration in the final 5-ml. volume used for the production of the blue color. In making a determination, a sample of milk is treated as described, and the percentage of light transmitted is found. Locating this figure on the curve in figure 1 will give the tyrosine equivalent in milligrams in a 5-ml. aliquot sample.

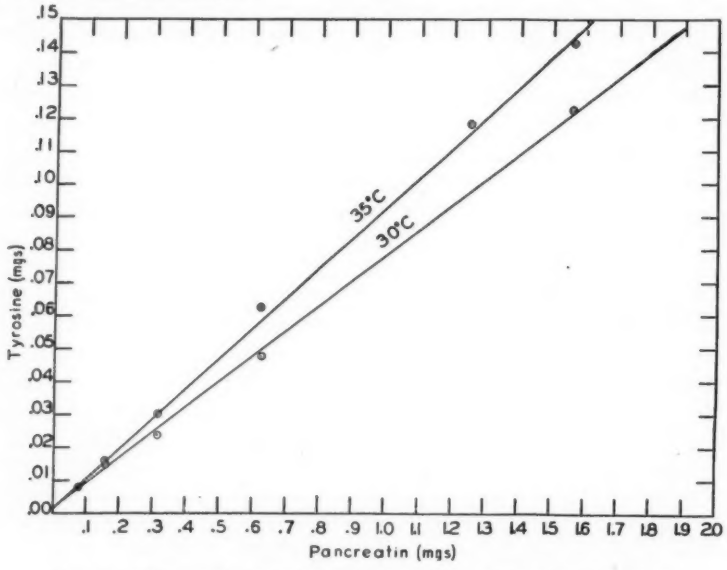

FIG. 2. Milk protein hydrolysis with pancreatic enzyme in 10 minutes.

CONCLUSION

A rapid colorimetric method can be used to measure minute amounts of milk protein hydrolysis. It is a very sensitive and a practical method for showing protein hydrolysis.

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STUDIES ON MILK PROTEINS. III. THE MODIFICATION OF MILK BY PANCREATIC ENZYMES

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The modification of milk by the use of pancreatic enzymes to give improved digestibility characteristics first was described by Conquest *et al.* (6) in 1938. Since that time, reports of clinical investigations (1, 2, 3, 4, 11), as well as results by various *in vitro* techniques (7, 10, 13, 14), have established the enzyme treatment as a suitable means of milk modification.

The active enzyme employed in the process is proteolytic and, if allowed to react for a sufficient period, will bring about pronounced protein hydrolysis. However, in the usual process, the enzymes are inactivated by heat after a relatively short time and the desired degree of modification is attained without altering the appearance or flavor of the milk or causing any substantial protein hydrolysis. The techniques common to protein study, such as the Kjeldahl analysis for total protein (6, 14), the determination of free amino acids by the Van Slyke method (13) or the formal titration (6), as well as studies of osmotic pressure (9) and conductivity (15), have shown no conclusive changes which would be associated with protein hydrolysis. Turner (13) did report, however, that the enzyme treatment caused an increase in the amount of protein soluble in 70 per cent alcohol. The lack of sufficiently precise techniques for studying protein degradation, as well as the fact that too little is known of the initial phases of enzyme activity, has made it difficult to offer a satisfactory explanation for the results obtained by enzymatic treatment.

A modified test for tyrosine, devised by Hull (8), has proved to be extremely sensitive for determining the breakdown of milk proteins. It seemed probable that this method could be applied to advantage in studying the enzyme treatment of milk and that it might make possible a more definite explanation of what had occurred. In the present experiment Hull's method was used as a means of determining protein degradation, with measurements of curd tension and protein stability included as criteria of the degree of treatment with pancreatic enzymes.

EXPERIMENTAL

Samples of enzyme-treated milk were prepared in the laboratory by adding appropriate amounts of a freshly prepared aqueous suspension of Enzylac Powder[1] to cold raw milk, heating the milk to 142° F. in 10 to 15

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[1] Pancreatic enzyme extract distributed by American Seal-Kap Corporation, Long Island City, N. Y.

minutes, holding at 142–145° F. for 30 minutes, and cooling in cold running water. Enzyme concentrations of 20, 40, 60, 80, and 100 p.p.m. were used in each series of samples; raw and pasteurized controls also were included.

Curd tension tests were performed in accordance with the method recommended by the American Dairy Science Association Committee on Curd Tension (5), using an American Curdometer.

The technique used for determining protein stability was that reported previously by the author (12), comprising briefly the addition of small increments of N/10 hydrochloric acid to 10-ml. portions of the sample in a series of test tubes, heating the tubes to boiling in a water bath, and then examining to determine the smallest amount of acid required to cause coagulation. The "Stability number" reported is equivalent to 100 times the ml. of N/10 acid used to produce coagulation.

Tyrosine tests were carried out as described by Hull (8). Color development was measured photoelectrically, using a Lumetron Model 402EF colorimeter with Corning Glass Filters no. 3962 and 2403 in the primary position. This filter combination has maximum transmission at a wave length of approximately 700 mμ, which is satisfactory for the purpose. Values for tyrosine were interpreted from a standard curve prepared from known tyrosine dilutions, using the same procedure as employed in the regular test. Amounts of tyrosine are expressed as "Tyrosine units", a unit being 1 mg. of tyrosine per liter of sample or its equivalent. The possibility of some other amino acids reacting in the test was considered, and a brief investigation indicated that the method was sensitive to tyrosine and tryptophane but not to cystine or phenylalanine. Considering tyrosine recovery in the test to be 100 per cent, tryptophane recovery was found to be approximately 88 per cent. Bearing in mind the relative amounts of these two amino acids in milk protein, it is believed that about 80 per cent of the blue color development in the test is due to tyrosine while 20 per cent is due to tryptophane. These figures are based upon empirical values and are cited in explanation of the use of tyrosine units as a means of expressing results.

RESULTS

Five series of samples were prepared. The average results of curd tension, protein stability, and tyrosine determinations are shown in table 1.

Statistical analysis revealed that, while both relationships were significant, tyrosine values were related more closely to protein stability than to curd tension. The following regression equations were obtained, with the correlation coefficients (r) as shown:

$$Y = 36.3 - 0.25x \qquad r = -0.936$$
$$Y' = 127 - 1.25x \qquad r = -0.963$$

in which Y = curd tension
Y' = protein stability number
x = tyrosine units

TABLE 1

Effect of added enzyme upon curd tension, protein stability, and tyrosine content of milk

(Average results—five series of samples)

Treatment	Curd tension	Protein stability no.	Tyrosine units
Raw milk	28.0	76	35.4
Pasteurized milk	27.0	82	36.2
Enzyme—20 p.p.m.	24.6	68	47.2
Enzyme—40 p.p.m.	22.4	56	57.2
Enzyme—60 p.p.m.	19.2	46	65.6
Enzyme—80 p.p.m.	18.2	36	73.4
Enzyme—100 p.p.m.	16.4	24	80.6

Av. time of heating to pasteurizing temperature was 13 minutes.

DISCUSSION

In view of the natural low curd tension of the raw milk which was available for experimentation, a feature not uncommon in the Chicago area, it would be unwise to attribute too much significance to curd tension relationships which were developed. The author feels that wider discrepancies might be found if milks of greater curd tension were examined, although the samples which were prepared were comparable with milks generally encountered in this market area. The value of curd tension as an index of the degree of enzymatic modification is lessened considerably in milk of this type, since the upper limit of 30 g. generally accepted in the industry for soft curd milk has little relationship to the treatment under such circumstances. Therefore, as an arbitrary index, it was felt that milk which had been processed in such a manner as to reduce the curd tension at least a third, or to about 20 g., could be considered as having been treated satisfactorily.

The effect of the enzyme action on the protein stability, on the other hand, offered a much more reliable index of treatment. Unpublished data by the author, including several years of practical experience with the commercial preparation of enzyme milk, have shown that satisfactory enzyme modification yields milk with a protein stability number in the range from about 20 to 45. Up to this time, the stability test has been the simplest and most convenient means of assaying treatment, although no claim has been made for it as an index of digestibility and it has served chiefly as a method of process control.

On the basis just described for deciding what constitutes satisfactory enzyme treatment, the data show that a curd tension of 20 g. corresponds to a tyrosine value of 65 units, whereas a protein stability number of 45 is equivalent to 65.6 tyrosine units. The correlation between the amount of tyrosine and these properties which have been used in the past as indices of enzymatic treatment suggests that the tyrosine value also might serve as

a measure of the degree of modification. In fact, the tyrosine value might well be a superior means of measurement, since it must be the direct result of protein degradation and, hence, a true measure of protein modification rather than an indirect or arbitrary index. Therefore, a tentative limit of at least 65 tyrosine units might be considered as a suitable minimum level for proper enzyme modification.

The average tyrosine value found in untreated milk, both raw and pasteurized, was approximately 36 units. Although large numbers of determinations have not been made, it has been unusual to find normal untreated milk with fewer than 30 tyrosine units or more than 42. In any case, satisfactory treatment of milk by the enzyme process has resulted invariably in the production of at least 30 additional units of free tyrosine in the sample. This, then, affords a basis for an estimate of the amount of enzymatic protein hydrolysis. By calculation from empirical values, the liberation of 30 additional tyrosine units represents about 1 per cent of the theoretically possible total of all tyrosine and tryptophane in the milk which might be freed in a complete hydrolysis. Therefore, it is not surprising that less sensitive methods of analysis have failed to show any significant changes as a result of mild enzyme activity.

The work of Keil and Roundy (9) and others (6, 13, 14, 15) would indicate that mild enzymatic action brings about no gross changes in the milk protein, from which it may be concluded that the protein molecule has not been altered materially. However, there is no doubt that this relatively mild treatment does affect other properties of the milk, such as curd characteristics, which generally are not considered as appropriate measures of hydrolysis, if related at all. The zone within which enzymatic modification is accomplished falls within the very earliest stages of protein breakdown.

The results of this investigation indicate that a significant increase in tyrosine, if not other amino acids as well, can be shown by a suitably sensitive technique. This may seem incompatible with prior results if it is believed that hydrolysis proceeds more or less simultaneously throughout the protein mass. However, the general concept is that proteolytic enzyme activity is a surface phenomenon, with the action not penetrating deeply into the molecule. Since the protein molecule is large and complex, the proportion of it engaged in a surface reaction at any one time would be small; it is quite likely that pronounced hydrolysis of the surface layer could occur without bringing about changes great enough to be detected upon subjecting the entire mass to analysis. Under this assumption the remaining portion of the molecule still would be in essentially its original condition, considering the composition of the mass, although the surface characteristics probably would have been altered. These surface changes would be expected to involve the degree of hydration as well as the electrical charge of the particles, properties which are of primary importance with respect to the stability and coagulating characteristics of the protein.

The theory is advanced that the effects of enzymatic modification of milk are due principally to the action of the enzymes upon the surface of the protein. This can account for the lack of gross changes in the protein as well as the evidences of slight hydrolysis. Furthermore, through changes in surface effects, the modifications in curd characteristics which have been observed can be explained.

SUMMARY AND CONCLUSIONS

A sensitive test for tyrosine has been applied to a study of milk modified by the enzyme process, with results indicating that the free tyrosine content of the milk increases with the degree of enzymatic treatment. The relationship seems to be sufficiently satisfactory for the tyrosine test to be given consideration as a new measure of enzymatic modification of milk and a tentative level of 65 tyrosine units is suggested as a suitable minimum limit.

Relationships of the free tyrosine content of milk to the curd tension and the protein stability were determined statistically and found to be significant. The degree of correlation was highest for the tyrosine-stability relationship.

According to the amount of tyrosine found in properly treated samples, it is estimated that normal enzyme modification of milk accounts for hydrolysis amounting to approximately 1 per cent of the theoretically possible total.

A theory is offered suggesting that changes brought about on the surface of the protein molecules by the action of proteolytic enzymes are responsible for the modified characteristics of enzyme-treated milk.

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THE ISOLATION AND PRELIMINARY STUDY OF SOME PHYSIOLOGICAL CHARACTERISTICS OF THE PREDOMINATING FLORA FROM THE RUMEN OF CATTLE AND SHEEP[1]

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The importance of microorganisms in ruminant digestion generally is accepted, but little is known about the physiological activities of the predominating flora. This fact is emphasized in Hastings' recent review (3). Some studies have been carried out to determine the role played by rumen bacteria in urea utilization (7, 9, 13, 15), and in cellulose digestion (5, 8, 10, 14).

Studies were initiated in 1945 to develop techniques for the culturing of the predominating bacteria in order that their physiological activities could be investigated. The successful procedures include: (a) a direct slide counting technique using an indirect stain, to serve as a guide for the success or failure of the cultural work; (b) a method for obtaining a sample suitable for culture from a fistulated animal; (c) an anaerobic procedure for growing the bacteria in the numbers indicated by the direct count; (d) methods for studying urea utilization; and (e) methods for cellulose breakdown.

EXPERIMENTAL RESULTS

Technique for slide counts. The direct counting technique found most successful involved the use of nigrosine, which colored the background but left the bacteria unstained. Rumen contents were diluted with 1 per cent glucose solution to 1:1,000 or 1:10,000, depending upon the expected bacterial content of the sample, and shaken for 5 minutes at a speed of 240 oscillations per minute. The vigorous shaking was very essential in order to free the bacteria from the food particles and break up clumps of bacteria. After shaking, 0.01 ml. of the sample was transferred by a standard loop to a thoroughly cleaned slide upon which an area of 2×2 cm. had been marked off. A similar loopful of one-half saturated methyl alcohol solution of nigrosine was mixed thoroughly with the sample, and spread evenly over the 4 cm.^{2} area. The slide was placed on a very hot electric plate so that drying occurred within 2–3 seconds. The slide now was ready to count, the bacteria appearing white against a black background.

Counts were made from several fields on three to five different slides,

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so that at least 400 cells were enumerated; the calculations were made according to Knaysi and Ford (6).

This technique was not intended to be extremely accurate but was employed in this study as a guide for the success or failure of the cultural results. The method used has the advantage of being rapid, and the counting is easy. It gives a permanent record of the organisms in the sample and overcomes difficulties encountered by other workers (7, 12). However, it has weaknesses. For example, both living and dead bacteria are counted, as they appear the same under the microscope. An attempt to overcome this difficulty by using a "vital" stain, following the procedure of Knaysi and Ford (6), did not appear satisfactory, as the bacteria died rapidly when exposed to air and some aeration could not be avoided in this technique.

Obtaining samples for cultural studies. The ideal subject for cultural work was found to be an animal with a fistula, since rumen contents could be obtained without undue exposure to air. Liquid samples from the rumen were taken by inserting a pipette into the bottom of the rumen, drawing up some of the liquid, and transferring it immediately into a stoppered flask nearly full of sterile 1 per cent glucose solution. The receiving flask was prepared by first autoclaving the glucose solution in a large flask and then transferring the solution to a sterile 1-l. flask which was filled nearly to capacity with 1,000 ml. of the glucose solution. After steaming for 1 hour, the flask was tightly stoppered and cooled rapidly in cold water. The cooled flask was taken to the experimental animal. The stopper was removed only long enough to receive the sample.

Solid samples of rumen contents were removed with a long-handled metal cup on which a tight-fitting rubber stopper was placed immediately after filling. One gram of this material was quickly weighed into a tared, sterile petri dish and transferred rapidly into a liter flask containing glucose solution.

Liquid samples taken by stomach tube and catheter from non-fistulated animals were studied by a similar procedure.

Procedure for culturing predominating flora. Success in culturing depended upon the continued maintenance of anaerobic conditions from the time of taking the sample until growth had occurred. From the rumen samples obtained, dilutions were made to 10^{-12}. Inoculations were made by pipetting the diluted sample into broth which previously had been placed for 10 minutes in a boiling water bath and then cooled rapidly to 45° C., immediately preceding inoculation. The tubes were sealed with vaspar directly after inoculation to maintain anaerobic conditions. Usually only the dilutions of 10^{-9} to 10^{-12} were inoculated, but occasionally 10^{-8} was included and 10^{-12} omitted. Speed is very essential in the whole procedure.

Several types of media were used in these experiments. Two media gave good results. A regular broth or agar had the following composition: 1 per

cent each of tryptone, peptone, beef extract, glucose, and yeast extract; 2 per cent fresh skimmed milk (added separately after removing medium from boiling water bath); and 0.5 per cent dipotassium phosphate. If agar were included, 1.5 per cent was used. The pH was 6.0.

A meat infusion broth plus sugar, a modification of the broth of Smith and Sherman (11), contained the following nutrients per liter: 1 g. glucose, 10 g. tryptose, 500 ml. meat infusion, 25 ml. tomato juice, and 5 g. dipotassium phosphate. The pH was 6.5–6.6. Both media usually had cellulose added in the form of acid-treated cotton, prepared as suggested by Hungate (4).

In almost every case in the high dilutions, the smear showed only one morphological type of organism. To obtain pure cultures for testing the physiological characteristics, an agar shake was made from the broth, and isolated colonies were picked.

Incubation of the cultures was at 38° C. and daily observations were made. Growth manifested itself, either by a general turbidity throughout the tube or by a change in the appearance of added cellulose, which was characterized by clumping or a tendency of the cellulose to cling together in strings. In either case a smear showing organisms confirmed the presence of growth. Ordinarily growth would appear in dilution 10^{-9} in 48 hours, but in two or three cases it was necessary to wait 60 days for growth to appear in the higher dilutions. Subcultures in agar shakes showed that the organisms isolated from the top dilutions were anaerobic, since no growth took place nearer than 0.25 to 0.50 inch below the surface.

Although the predominating cultures isolated usually compared with the morphology of the predominating organisms as seen on the original slides from the rumen contents, in some instances cocci which seldom were isolated were present in large numbers. This would indicate that the cultural technique should be improved, probably in respect to maintenance of anaerobic conditions, because the cocci apparently would survive less aeration than some of the rods.

Table 1 summarizes the data showing the average bacterial slide counts and the highest dilution in which growth was observed from samples obtained by various methods from animals on a normal diet. The slide count figures reveal no essential differences between sheep and cattle in the bacterial population of their rumen contents. The solid samples from the fistulated animals of both species showed about the same average number of bacteria per gram, and the samples obtained from slaughtered sheep by forcibly squeezing the liquid out of the solid were similar to the figures for solid samples. Probably these latter samples more nearly represented a solid sample than a liquid one, due to detachment of bacteria which normally would adhere to the solid material. In both species the number of bacteria was somewhat lower in the liquid than in the solid samples.

TABLE 1

Comparison of average bacterial slide and cultural counts from samples of rumen contents obtained by various methods from animals on a normal diet

Sample	Type	No. of samples	Av. slide count	Av. slide count of samples in highest dilution showing growth			
			(*billions per g. or ml.*)	(*below 10-9*)	(*10-9*)	(*10-10*)	(*10-11*)
Cattle with fistula	Liquid	10	76 ± 10.3*	None	29 (1)†	64 ± 8.7 (5)	100 ± 15.5 (4)
	Solid	16	92 ± 8.3	None	29 (1)	76.5 ± 10.7 (4)	110 ± 7.9 (11)
Sheep with fistula	Liquid	4	62 ± 9.3	53 (1)	47 (1)	60 (1)	89 (1)
	Solid	4	93 ± 19.5	135 (1)	80 (1)	80.5 ± 32.5 (2)	None
Sheep by stomach tube	Liquid	16	27 ± 2.23	27 ± 7.2 (3)	27 ± 7.8 (11)	30.5 ± 3.5 (2)	None
Sheep slaughtered	Liquid expressed from solid	9	95 ± 5.7	.....	.....	.....	.....

* Standard deviation.
† Numbers in parentheses indicate number of observations.

Athough the individual counts of the liquid and solid samples show considerable overlapping when both types of samples were obtained from the same animal at the same time, the bacterial count of the solid always was higher than that of the liquid sample except in one case, where they were equal. This would indicate that the liquid really did have a somewhat lower bacterial population than the solid.

It also can be seen from table 1 that the method of obtaining the sample was important. The liquid samples obtained by stomach tube gave a lower average slide count than the liquid samples obtained from fistulated sheep by pipette, which would indicate that these samples taken by stomach tube were not representative even of the liquid fraction of the rumen contents. Differences in animals and diets also may have influenced the results.

Data also are presented (table 1) which show that there is good agreement between the slide and cultural "counts" on samples obtained from the fistulated bull. In 24 out of 26 cases the samples grew in the dilutions expected from the slide count. This would indicate that success had been achieved in culturing the predominating organisms. However, such good agreement was not obtained with the fistulated sheep since in only three cases out of eight did the slide and cultural results agree. These results were better than those obtained with samples taken from the sheep by stomach tube, where only 2 out of 16 samples showed growth in the dilution indicated by the slide count.

As stated above, the maintenance of anaerobic conditions was essential for good cultural results, since most of the rumen organisms are anaerobic and the close agreement between the slide and cultural results obtained with the fistulated bull reflect the good cultural conditions available when working with this animal. The poorer agreement between the slide and cultural counts with samples from the fistulated sheep probably is due to the less favorable conditions prevailing in sampling the sheep. The aeration attendant upon taking a stomach tube sample would account for the poor results obtained in culturing this material. Aspirated samples appear poor because they did not give representative samples of the rumen contents and they permitted aeration of the material.

The slide and cultural counts found in this study are higher than any figures reported in the literature. The highest slide count reported was about 13 billion organisms per gram of fresh contents (7), while the average obtained in this study from the solid material from a fistulated animal approximated 100 billion organisms per gram. The highest cultural growth was reported by Hungate (5), who obtained growth of cellulose-digesting bacteria from rumen contents diluted one billion times when cultured in inorganic medium. In the present study bacteria, including many cellulose digesters, have been grown from a 1:100 billion dilution of rumen contents. The figure of 100 billion bacteria per gram of fresh rumen contents

agrees well with the calculated figure of 100 billion bacteria as cited by Köhler (7). Further evidence in support of the probable accuracy of the counts reported here is the satisfactory agreement between the slide and cultural counts.

Test for urea utilization and cellulose digestion. To test for urea utilization the organisms isolated from dilutions 10^{-10} and 10^{-11} were inoculated into an inorganic salt broth (4), modified to contain 1 per cent glucose, 0.04 per cent urea (in place of ammonium sulfate), 20 μg. of each of the following vitamins—thiamine, riboflavin, pyridoxin, pantothenic acid, niacin, and paraaminobenzoic acid; and 0.05 μg. of biotin. The mixture of vitamins was autoclaved separately and added aseptically to each tube at the time of inoculation. The pH of the broth after all additions was 6.5. In order to produce and maintain anaerobic conditions, the tubes were treated and sealed as previously described. If the cultures survived three transfers in this broth, it was assumed that they were able to utilize urea.

In the early part of this work, in order to determine whether urea actually had disappeared in those cultures in which growth had occurred, determinations of total nitrogen and of urea were run on several broths which had been inoculated with low dilutions (10^{-3} and 10^{-4}) of rumen contents from lambs fed 40 per cent of their protein equivalent as urea. An uninoculated blank tube also was tested.

The determination of total nitrogen was carried out by the usual colorimetric procedure, digesting 1 ml. of the medium with acid digestion mixture and Nesslerizing. The urea was determined by the urease method on 1 ml. of the medium. This also was Nesslerized. All analyses were run in duplicate.

Some of the cultures isolated from 10^{-10} and 10^{-11} dilutions of rumen contents were tested for cellulose digestion by a modification of the technique of Fuller and Norman (2). The cultures were inoculated into the meat infusion broth prepared as previously described, except that only 0.01 per cent of glucose was included. The cultures were incubated from 2 to 4 weeks in a desiccator which had been evacuated and filled to one-half capacity with carbon dioxide repeated three times. All cultures were run in duplicate and the results of the two tests were averaged.

When incubation had been finished, the supernatant fluid was decanted through a tared Gooch crucible, filtered by suction, and the pH tested. The residual cotton in the test tube was treated with 10 ml. of 0.1 per cent sodium carbonate for 5 minutes to remove the bacterial gum which some cultures formed in the presence of glucose. Several washings were carried out with small portions of distilled water, followed by treatment with 1.0 per cent acetic acid and several more washings with distilled water. All the washings, including the sodium carbonate and acetic acid, were poured through the crucible. The crucibles were then dried for 16 hours in an oven at

100° C., and weighed to determine cellulose loss. Uninoculated blanks were carried through the entire procedure, and in all determinations the same lot of cellulose was used.

Although only 20 mg. of cellulose was used per tube, the accuracy of the results obtained with the blank determinations (where recoveries ranged within 0.2–0.5 mg.) seemed to indicate that any loss over 5 per cent would be significant. Actually in only one case was a value as low as 7.0 per cent loss considered as positive. Duplicates tested on the same or on different days gave essentially the same results. The figures showing cellulose loss are too low, as the weight added by bacteria clinging to the cellulose fibers and not removed by washing actually increased the weight of the recovered cellulose.

Under conditions in the rumen, the degree of cellulose decomposition brought about by these bacteria probably is greater than that shown by the *in vitro* tests, where no agitation of the cellulose took place.

Some of the predominating organisms were tested for their ability to utilize urea and to break down cellulose.

Seventeen cultures from dilutions 10^{-10} and 10^{-11} of the rumen contents from a fistulated bull fed a normal diet were tested for cellulose digestion and 12 of these also were studied for their ability to utilize urea. Of these organisms, 15 were rods of varying morphology and two were cocci. All of the cultures tested were found to be Gram-positive, non-motile organisms which produced acid, frequently giving a pH as low as 4.5 in the broth in which they were growing. One coccus and one rod produced slight amounts of gas. Ten out of 12 cultures tested utilized urea and 12 out of 17 broke down cellulose. The majority decomposed 10–20 per cent of the added cellulose, which agreed with the results obtained by Cowles and Rettger (1). One coccus broke down 28 per cent which was higher than any of the rods tested.

Twenty-three cultures isolated from 10^{-10} and 10^{-11} dilutions of rumen contents from sheep fed normal and experimental diets were tested for their ability to utilize urea and break down cellulose. All of them were rods and all of those tested for the following characteristic were Gram-positive organisms. Only one culture was motile. They produced a final pH of about 4.5–5.0 in broth, and only two cultures produced gas. Seven of 17 cultures tested for urea utilization were positive and only 10 out of 23 cultures showed breakdown of cellulose. Again the majority of cultures decomposed 10–20 per cent of the added cellulose, while one broke down 48 per cent.

In agreement with earlier results of Pearson and Smith (9), the analyses to determine whether urea actually was utilized by the organisms showed that 6–21 per cent of the urea had been lost from the broth. The total nitrogen remained the same in all of the tubes, except in two cases, where small amounts of gas had been produced and had escaped.

Because of the small series of tests conducted and differences in diets fed the two species, no conclusions should be drawn about any differences between the species with respect to these two characteristics.

All the samples from fistulated cattle were from one young bull. Although these are preliminary studies, it would seem that the techniques described could be used as a basis for further work in this field.

SUMMARY

1. New techniques for studying the microbiology of the rumen are described. These include a direct slide count technique, a cultural procedure for isolating the predominating flora, a method for obtaining a sample under anaerobic conditions, and methods for the determination of urea utilization and cellulose digestion by the predominating bacteria.

2. The direct slide count technique made use of nigrosine, a negative stain, which reduced to a minimum inaccuracies caused by artifacts.

3. When good cultural conditions prevailed and a fistulated animal was used, slide and cultural counts agreed closely. Material obtained by stomach tube gave consistently lower bacterial counts than samples taken directly from a rumen fistula. To isolate the predominating flora, the most important feature of the cultural technique was the maintenance of anaerobic conditions from the time of sampling until growth was obtained. A rich organic medium with a pH of 6.0 was used and cultures were incubated at 38° C.

4. It was shown by these methods that bacteria are present in the rumen contents of both sheep and cattle in numbers of about 100 billion per gram of fresh material.

5. Bacteria isolated from the highest dilutions of rumen contents from both sheep and cattle were tested for their ability to utilize urea and break down cellulose, and many of these cultures proved capable of performing these functions *in vitro*.

6. A few of the bacteria isolated from the highest dilutions of rumen contents were studied and were found to be mostly Gram-positive, non-motile anaerobic rods. A small number of cocci were cultured.

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MILK LIPASE SYSTEM. I. CHEMICAL ANALYSIS OF FAT, WASHED FAT, AND THE ALCOHOL SOLUBLE FRACTION OF FAT OBTAINED FROM HOMOGENIZED RAW MILK[1]

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Considerable question yet exists as to the specific fatty acids which are involved in lipolysis of milk fat. Davies (2) reports that 60–70 per cent of the increase in the titer of milk fat which results from lipase action is due to oleic acid. On the basis of distillation studies, Hiscox and Harrison (5) and later Gould and Johnson (3) concluded that the fatty acids liberated by lipolysis were largely of the water-insoluble, volatile type. Kelly (8) reported milk lipase to exhibit selectivity by acting more rapidly on the volatile than on the non-volatile substrates.

Only meager attention has been given to the use of chemical analysis of milk fat as a means of ascertaining the effects of lipase action. In a previous study (4), fat from non-rancid and rancid milk was found not to differ appreciably in Reichert-Meissl and Polenske numbers and in the refractive index, even though the rancid fat exhibited a greatly higher acid degree. One limitation of this study, however, was that in the case of the rancid fat, the freed fatty acids remained with the unhydrolyzed portion to be included in the various determinations. Thus, the presence of the free acids may have prevented a true differentiation between the rancid and non-rancid fat by the methods used. In view of this possibility, it seemed desirable to conduct additional studies of chemical changes in milk fat resulting from lipolysis. Procedures were utilized which permit the separation of the free fatty acids from the rancid fat before the fat characteristic determinations are conducted. The results of such studies constitute the basis for this paper.

EXPERIMENTAL PROCEDURE

Mixed-herd milk from the University dairy farm was used in these experiments. Rancidity was produced as desired by homogenizing the raw milk with a low-pressure centrifugal homogenizer and then storing it for several hours at about 35° F. before pasteurizing. The purified fat for subsequent treatment and analyses was obtained from the milk by the standard procedure of separation, churning, and filtration. Filtration was conducted by melting the butter granules and then filtering the butter oil through paper in an incubator maintained at 55° C.

Fat titrations were conducted by the procedure described previously

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(3), and results are expressed as acid degrees (ml. of N NaOH per 100 g. fat). The determinations of all other fat characteristics were made in accordance with official methods of the A.O.A.C. (1).

Two different procedures were used in preparing the fat or its extract for chemical analyses. These are designated as the washing procedure and the alcohol extract procedure.

The washing procedure. This procedure was essentially the same as that described previously (3) as based on the work of Hiscox *et al.* (6), and consists of washing portions of a milk fat–ether mixture with successive lots of weak alkaline solution, removing the retained alkali by washing several times with water, and then drying the fat by means of a hot plate and a 100° C. oven. Various fat characteristics were determined on the fat before and after the washing procedure. One difficulty of this procedure was the susceptibility of the mixture to the formation of an emulsion during the washing with the alkaline solution.

The alcohol extraction procedure. Preliminary trials revealed that an extraction procedure involving hot ethanol for separating the freed fatty acids from the fat could be used with greater efficiency and rapidity than the alkali washing procedure. The following procedure was developed as a result of these preliminary observations.

Sixty grams of butter oil were heated to boiling in 120 ml. of ethanol, the hot mixture centrifuged, and the supernatant ethanol layer largely removed by siphonation. The treatment with hot ethanol was repeated twice, and all of the lots of extractant combined. Following the last centrifuging, the lower fat layer was congealed by placing the container in ice water; then the alcohol layer was decanted.

The alcoholic extracts from two lots of fat were combined, cooled, and held overnight at 32–35° F. A copious white precipitate which formed during this cold storage was removed by filtration in the cold room, and was washed with several portions of cold ethanol at the same temperature. The washings were combined with the filtrate.

The filtered alcohol solution then was heated under a partial vacuum to remove the alcohol. About 30–40 minutes usually were required for removing the alcohol from 120 g. of fat, and the heating temperature generally was under 50° C. The final alcohol extract was subjected briefly to hot plate heating at 100° C. to insure drying and then was held in a desiccator under vacuum until analyzed.

EXPERIMENTAL RESULTS

The alkali washing procedure. The effect of washing rancid and nonrancid fat with the weak alkaline solution is revealed by the data in table 1, which shows changes in acid degree, Reichert-Meissl number, Polenske number, and iodine number (Hanus).

These results show that only in the case of the acid degree is there a distinct relationship between the extent of lipolysis and the change resulting from the alkaline washing. This lowering of the acid degree is to be expected. However, the data do indicate a tendency for the washing procedure to increase the Reichert-Meissl number and decrease the iodine number, but the changes are slight and are no more pronounced for the rancid fats (lots 2, 3, 4) than for the non-rancid fat (lot 1).

On the basis of the results in table 1, the particular procedure used apparently is unsatisfactory as a means of determining the fat changes, other than acidity, which result from lipase action. Because of the failure of the washing procedure to yield satisfactory results, attention was diverted to the alcohol extract procedure.

TABLE 1

The influence of alkaline washing of non-rancid and rancid fat on certain fat characteristics (av. 3 trials)

Lot no.	Treatment of sample	Fat characteristics			
		Acid degree	Reichert-Meissl	Polenske	Iodine (Hanus)
1	Non-washed	0.4	28.4	1.6	36.7
	Washed	0.2	28.7	1.5	36.4
2	Non-washed	3.9	28.7	1.6	36.8
	Washed	0.5	28.7	1.5	36.5
3	Non-washed	4.4	28.3	1.5	36.8
	Washed	0.7	28.6	1.6	36.4
4	Non-washed	4.8	28.3	1.6	36.2
	Washed	0.9	28.7	1.7	36.0

The alcohol-extract procedure—preliminary observations. Before this procedure was devised completely, various preliminary experiments were conducted. One series of these preliminary trials pertained to the efficiency of alcohol in removing the free fatty acids. In this series, 60 g. of rancid fat were heated to boiling in 120 ml. ethanol, centrifuged, and the lower layer of fat congealed in ice water. The top layer, *i.e.*, the alcohol portion, was decanted and titrated. The fat layer then was freed of remaining alcohol by heating on a hot plate, and a sample of the fat removed and titrated. Three additional extractions were made, with titrations of the alcohol extract and the remaining fat being made in each case. Results are presented in table 2.

These data reveal that in the case of this highly rancid fat, three washings removed more than 90 per cent of the free fatty acids as determined by titration of 10-g. samples, and four washings removed more than 95 per cent of these acids. The heat treatment of the fat in the alcohol in itself did not alter the titer, indicating that acidity decreases due to esterification are negligible, if they occur at all.

TABLE 2

The effectiveness of hot ethanol extraction in removing the free fatty acids from butter oil

No. of alcohol extractions	Titer of ethanol extract*	Titer of 10 g. fat samples	Per cent of original acidity in fat
	(*ml. N/20 NaOH*)	(*ml. N/20 NaOH*)	
0	.....	22.9	100.0
1	81.9	10.2	44.5
2	24.0	4.8	21.0
3	7.8	2.1	9.2
4	1.9	1.0	4.4

* Corrected for titer of the alcohol.

Naturally, the alcohol treatment of butter oil not only removes the major portion of the free fatty acids present but also all lipoid material which is soluble in hot alcohol. For example, a 60-g. sample of fat having an acid degree of 11.4, extracted with four successive 120-ml. lots of hot alcohol, showed a total loss in weight of about 17 g., only a portion of which would represent the free fatty acids. Furthermore, observations indicate that only a comparatively small portion of this alcohol-soluble fraction precipitates from the alcohol during overnight storage at 32–35° F. and that this precipitate does not retain an appreciable amount of the free fatty acids. In two typical trials utilizing rancid fat, the clear alcohol solution obtained following overnight storage and filtration required 119.8 and 116.2 ml. of N/10 NaOH, respectively, whereas the corresponding precipitates which were removed by the filtration required but 0.8 and 0.6 ml. N/10 NaOH.

In general, the yield of lipid extract finally obtained for analysis varied directly with the titer of the original fat. For example, three lots of non-rancid fat having an average acid degree of 0.9 yielded an average of 5.8 g. of extract per 100 g. of fat, whereas three lots of rancid fat with an average acid degree of 10.0 yielded an average of 11 g. of extract per 100 g. of original fat.

TABLE 3

Certain chemical characteristics of non-rancid and rancid fat and of their ethanol extracts (av. 3 trials)

Characteristic	Non-rancid			Rancid		
	Fat	Extract	Difference: extract less fat	Fat	Extract	Difference: extract less fat
			(%)			(%)
Acid degree	0.55	5.2	+845.4	10.5	64.5	+514.3
Reichert-Meissl no.	27.8	56.3	+102.5	26.7	43.7	+ 63.7
Polenske no.	1.8	4.1	+127.8	1.7	3.2	+ 88.3
Saponification no.	227.4	249.3	+ 9.6	226.2	238.3	+ 5.3
Iodine no.	35.1	38.3	+ 9.1	35.9	38.3	+ 9.5
Refractive index	1.4544	1.4535	− 0.06	1.4546	1.4528	− 0.1

The extract obtained was a normal golden yellow color and was liquid at room temperature. The flavor of the fat from non-rancid cream was not rancid in flavor, although it retained the flavor of ether to some extent. The extract from the rancid cream possessed an extremely pungent, rancid, bitter flavor.

Several characteristics of non-rancid and rancid fat and their respective alcohol extracts are presented in table 3. These data reveal that in comparison to the original fat, the extracts are higher in acid degree, Reichert-Meissl and Polenske numbers, saponification number, and iodine number, but lower in refractive index. The differences between the characteristics of the fat and its extract are especially pronounced with the acid degree and the Reichert-Meissl and Polenske numbers. In the case of the non-rancid series, the extract (when compared to the original fat) exhibits a two-fold increase in Reichert-Meissl number, a 2.3-fold increase in Polenske number, and a 9.4-fold increase in acid degree. In the rancid series, the acid degree, the Reichert-Meissl, and the Polenske are greater in the extract than in the fat by 6.1-fold, 1.6-fold, and 1.9-fold, respectively. On a percentage basis, the differences between these three characteristics for the fat and extract are less in the rancid series than for the non-rancid series.

The results in table 3 also reveal that the difference in saponification number between the fat and the extract is less for the rancid than for the non-rancid series, whereas the change in iodine number is substantially the same.

DISCUSSION

The results obtained reveal the possibility of utilizing alcohol extraction of milk fat as a simple means of concentrating the fatty acids liberated by lipase action. Also, this alcohol extraction procedure would appear to create conditions more favorable for specific identification of the acids involved since it separates the fatty acids from the major portion of the milk fat.

Even though the alcohol extraction resulted in a portion of milk fat more highly concentrated in free fatty acids than the original fat, the chemical analysis of the extract by the methods used in this study does not appear to yield data sufficiently specific to offer a substantial basis for conclusions relative to the selectivity of the milk lipase system for certain acids or groups of acids. The fat characteristics of the alcohol extracts did reveal a high concentration of the shorter-chained fatty acids, but this occurred in both the non-rancid and rancid series. It is true that on a percentage basis, the concentration of shorter-chained fatty acids did not differ as much between the rancid fat and its extract as between the identical pair in the non-rancid series, whereas the iodine numbers were essentially identical. On this basis, the extract from the rancid fat may be said to contain a higher

percentage of saturated, non-volatile, non-soluble fatty acids than the corresponding extract from the non-rancid fat. However, it would appear illogical to draw any definite conclusions in this connection without additional and more specific evidence.

One factor which may affect the results obtained in a chemical analysis of milk fat is the method of preparing the fat. The fat used in this study was prepared by the conventional churning procedure. More recently (7) it has been demonstrated that extracting the fat from milk or cream will yield a product appreciably higher in free fatty acids. Subjecting this extracted fat to chemical analyses may reveal more concerning lipolytic effects than has been achieved in this study on fat obtained by churning.

In general, the use of fat characteristics to distinguish between a non-rancid and a rancid fat apparently leaves much to be desired, even though the free fatty acids present are removed first. However, the failure of the analyses to reveal a high degree of lipase selectivity should not necessarily imply that this is due entirely to a lack of sensitivity in the methods used. More logically, one may accept these results as offering some basis for the assumption that milk lipase acts rather generally on all of the glycerides present and to about the same extent, thus yielding a mixture of free fatty acids which, chemically, resemble quite closely the original fat. It is true, however, that a more specific identification of the fatty acids involved is necessary before final conclusions may be drawn in this connection.

SUMMARY AND CONCLUSIONS

Studies were conducted to ascertain the possibility of determining the specific fatty acids involved in lipase action in milk by applying chemical analyses (a) to milk fat before and after removing the fatty acids by alkaline washing and (b) to an extracted product obtained by treating milk fat with hot alcohol. Acid degree, Reichert-Meissl number, Polenske number, iodine number, and refractive index were used.

Other than in acid degree, the chemical analysis of milk fat before and after the alkali washing did not reveal any distinct difference between non-rancid and rancid fat.

The alcohol extraction method resulted in a fraction in which the free fatty acids were highly concentrated and which also contained a greatly increased proportion of the lower fatty acids in comparison to the original fat. However, the Reichert-Meissl, Polenske, and iodine numbers and the refractive index of the alcohol-soluble fraction were not sufficiently different between the rancid and non-rancid series to reveal any appreciable specificity of the lipase action for particular fatty acids.

A brief discussion is presented of the limitation of the results and of the interpretations which may be placed thereon.

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ABSTRACTS OF LITERATURE

BACTERIOLOGY

387. Microscopical Examination of Milk. D. B. Shutt. Canad. Dairy and Ice Cream Jour., *26*, 6 : 32. June, 1947.

A method has been developed whereby the milk smear can be defatted, fixed, and stained in one operation in from 5 to 15 seconds. During this process the background is completely decolorized while the bacteria and cells are stained blue. The method is based upon two formulas using ether, absolute methyl alcohol, methylene blue, and hydrochloric acid. The solution is held in a stoppered bottle and in a refrigerator when not in use. The solution should be filtered before using. H.P.

388. The Effect of Heat-Resistant Reducing Substances on the Dye Reduction Tests Used for Estimating the Hygienic Quality of Pasteurizing Milk. M. A. Edwards and C. S. Morris. Starcross, Devon, Eng. Dairy Indus., *12*, 7 : 648. July, 1947.

The effect of heat-resistant reducing substances produced by bacteria in milk upon the methylene blue and resazurin tests applied to the milk after pasteurization was studied. Different types of organisms were grown in sterile milk for various periods. The milk was pasteurized and then subjected to the tests immediately and after 24 and 48 hr.

The amount of reducing substances formed appeared to vary with the development of acidity. These substances gradually lose their reducing properties on aging the pasteurized milk. From a practical point of view, the effect of the heat-resistant reducing substances in pasteurized milk probably is quite unimportant, since milk supplies having significant quantities of these substances also would have very high acidity and would be rejected at the receiving platform. D.V.J.

BREEDING

389. Heritability of Amount of Spotting in Holstein-Friesian Cattle. P. Briquet and J. L. Lush, Iowa State College, Ames. Jour. Hered., *38:* 99–105. 1947.

Three hundred forty-three animals by 24 different sires were studied. The repeatability of visual estimates made at different ages averaged 0.982. The possibility of the slight decrease in amount of white with age as being due to the changing proportion of leg area to body area is suggested. Sex appeared to have no influence on the amount of white.

Heritability estimates were determined by intrasire regressions of offspring on dam and by regression of the amount of white on offspring on average amount of white on parents. With estimates of 99 and 93%, respectively, obtained by these 2 methods, it is concluded that the heritability of individual differences probably is more than 90%. It is suggested that several pairs of genes are required to explain the results obtained. L.O.G.

390. **Cattle Breeding in India.** F. B. Ogilvie, American Delhi Military Area Command. Jour. Hered., *38:* 23–28. 1947.

The work of the Imperial Agricultural Research Institute at New Delhi in developing milk production in the Sahiwal and Tharpakar breeds is considered to make these breeds valuable to southern U.S. because of the climatic similarities of this area to the native homes of these cattle, Punjab and Sind, respectively.

Characteristics of these breeds are absence of dystocia, birth weight of 50 lb. with males slightly heavier than females, milk testing 5% fat, longevity, calving at 28.5 months, and absence (or low incidence) of mastitis. The management practices include a calving interval aimed at 13 months, prenatal (prepartum) milking, calves separated from dams at birth, and 4× milking. A line breeding program is being followed. Photographs show the get of a bull whose daughters each produced 6,000–7,000 lb. of milk during their first lactation and a string of cows each of which produced over 8,000 lb. of milk.

Foot and mouth disease always is present and an attack may ruin the productive life of a cow. The chief disease problems center around bipolar infection, calf pneumonia, and female sterility. There is no brucellosis in either herd. L.O.G.

391. **Artificial Insemination as a Means of Transmission of Bovine Venereal Trichomoniasis.** D. E. Bartlett, K. G. Teeter, and P. C. Underwood. Amer. Vet. Med. Assoc. Jour., *111,* 845: 114–116. Aug., 1947.

The possibilities of transmission of Trichomoniasis from infected bulls to uninfected cows by means of artificial insemination is discussed. Of 9 uninfected females inseminated with fresh, undiluted semen collected from an infected bull by means of an artificial vagina, 6 became infected as a result of 7 inseminations; 3 did not contract the disease after 6 inseminations. T.M.L.

392. **Duration of Sperm Fertility in the Ewe.** W. W. Green. Amer. Jour. Vet. Res., *8,* 28: 299–301. July, 1947.

Fifty-five grade Shropshire ewes and 2 highly fertile grade Shropshire

rams were used in this experiment. The ewes were bred from 0 to 54 hr. after the end of estrus. The ewes that did not return in heat were slaughtered and the fetuses recovered. The ewes returning to heat were counted as non-pregnant.

The data reveal that sperm may retain the ability to fertilize an ovum after approximately 2 days' residence in the reproductive tract of the ewe.

T.M.L.

BUTTER

393. **The Titratable Acidities of Cream and the pH of Commercial Butter.** A. H. WHITE. Canad. Dairy and Ice Cream Jour., *26*, 6: 21. June, 1947.

The neutralizing data of 92 commercial and 75 semi-commercial churnings showed that the pH of butter can be maintained between the recommended range of 6.7 to 7.2 by accurate neutralizing procedures. From 75 to 82.5% of the churnings of 3 creameries analyzed over a period of a year were within this range. For cream with an initial acidity of 0.25–0.3%, the acidity should be reduced to 0.11–0.12%. In general, for each 0.1% increase in the initial titratable acidity, the desired acidity after neutralization should be increased by 0.01%. Variations in the pH of butter from any one plant were due to inaccuracies in determining the initial acidity of the cream, in estimating the weight of cream, and in calculating the amount of neutralizer to use.

H.P.

394. **Standardizing the Composition of Butter.** R. W. BROWN. Canad. Dairy and Ice Cream Jour., *26*, 6: 26. June, 1947.

A simple and accurate method of determining the amount of water to add to a churning of butter in order to standardize its composition is to make use of the "Pearson Square" method used in standardizing milk and cream.

H.P.

395. **Butter Boxes.** R. W. BROWN AND T. L. FORSTER. Canad. Dairy and Ice Cream Jour., *26*, 5: 31. May, 1947.

Examination and analysis of 7 boxes of Australian and New Zealand butter revealed that much paper fiber is used in the fabrication of the boxes. The all-fiber boxes showed up the best from the standpoint of flavor and sediment in the butter surfaces.

H.P.

396. **The Power Butter Printer.** J. C. PALMER AND P. J. BOGAERTS. Canad. Dairy and Ice Cream Jour., *26*, 7: 28. July, 1947.

In printing butter with a power printer it is important to have the butter evenly tempered throughout. The butter should be well worked and show

no free moisture. If printed too cold, some loss in moisture will result, accompanied by off color in the print. Streakiness can be overcome by: (a) not allowing the machine to operate unnecessarily with butter in the hopper; (b) feeding the machine evenly with even pressure; (c) printing butter between 37 and 47° F.; (d) in the case of twin auger machines, setting the edges of the spiral so that they meet when they turn; and (e) holding butter in the refrigerator for at least 24 hr. before printing. H.P.

397. Paper Board Flavor on Print Butter. D. B. SHUTT. Canad. Dairy and Ice Cream Jour., *26*, 7: 27. July, 1947.

Butter prints packed in unlined cartons developed a very disagreeable flavor, which was variously described as disinfectant, iodine, and even as chloride of lime. Corrugated paper cartons that are to be used for holding print butter always should be lined with waxed or other waterproof paper. H.P.

CHEESE

398. The Pink Test. I. HLYNKA, E. G. HOOD, AND C. A. GIBSON. Canad. Dairy and Ice Cream Jour., *26*, 5: 25. May, 1947.

A new test is proposed for determining setting time in Cheddar cheesemaking. It depends upon the reduction of resazurin to its pink end-point and is called the "pink test". One milliliter of standard resazurin solution is pipetted into a test tube to which a 10-ml. quantity of milk from a well-stirred vat is added. The tube is held at 86° F. and examined at the end of 5 minutes or until the original color is reduced to pink. The vat is ready to set when this color change takes place in 5 minutes. Cheesemaking experiments using starter at different levels were carried out in order to test the performance of the pink test. The pink test is based on a sound principle; it measures the rate of lactic acid development in the vat by measuring its rate over a short interval in cheese milk. H.P.

399. Cheese Making from Milk Pasteurized at High Temperatures. E. ANDERSEN, Danish Government Experimental Dairy Station, Hillerød, Denmark. Nordisk Mejeri-Tidsskrift, *12*, 9: 177–179. 1946.

Cheesemaking from milk pasteurized at high temperatures was tried for the first time more than 50 years ago in Denmark, but without success. Up to 1946 the method still proved unsuccessful.

The 6th annual publication from the Danish Government Experimental Dairy Station deals with the problem in connection with testing of a "Spirala" pasteurizer for cheese milk. "Danish Swiss cheese" has been made from milk pasteurized at 80° C. (176° F.) and 90° C. (194° F.). By chang-

ing the cheesemaking procedure for cheese made from milk pasteurized at high temperatures, cheese of a fair quality could be made as compared with cheese made from milk pasteurized at 70° C., flash-pasteurization. Holding times of 21 and 65 seconds were used. The higher temperature and the longer holding time resulted in the greater yield. Yield increases ranged from 4% at 80° C. held for 65 seconds up to 10% at 90° C. held for 65 seconds. The latter cheese was likely to be sour. Cheese made from milk pasteurized at high temperatures ripens very slowly. It is doubtful if the increased yield compensates for cost of pasteurization and reduction in quality.

Heating of milk to 80° C. or above causes changes in the colloidal calcium salts and coagulates the albumin. As a result of the changes in colloidal calcium salts, the coagulation of the milk by rennet is reduced considerably and the curd formed is abnormal. The precipitation of the albumin makes it difficult to get the whey out of the curd, and the cheese develops a short and brittle consistency so the grains will not stick together. The cheese will come from the press with a greater whey content than normal and consequently a higher lactose content. The pH of such cheese will have a tendency to be too low, the cheese becoming sour. The precipitation of the albumin is responsible for the greater yield, as the curd binds more water and the albumin goes into the cheese instead of into the whey.

Coagulation of the milk can be helped considerably by adding calcium chloride to the milk. Up to 30–50 g. per 100 kg. (220 lb.) of milk can be used. If the temperature is raised 2–3° C. (normal setting temperature, 86° F.), an almost normal curd can be obtained. Sour cheese can be prevented by using less starter, but the best way is to raise the cooking temperature and increase the amount of water used for heating purposes.

"Danish Swiss cheese", Port Salut, and other kinds of cheese of the "Tilsiter" type can be made from milk pasteurized at high temperatures, but the method is not good for Gorgonzola, Blue cheese, and Swiss. T.K.

400. Further Studies on the Measurement and Control of Physical Factors in Cheese Making. MARGARET BARON. Dairy Indus., *12*, 6: 548. June, 1947.

Two studies, one on Cheddar cheese and the other on long-keeping Cheshire cheese, were conducted. The physical properties of the cheese at different points of manufacture were correlated with the properties of the finished cheese. Bowl, ball compressor, and borer techniques were employed.

The bowl readings taken on the curd just before cutting appear to be closely related to the properties of the finished cheese. Numerous correlations were run involving such factors as milling acidity, milling time, solids-not-fat, and butterfat content. Tables showing the correlation coefficients developed from the data are presented. D.V.J.

401. Packaging Natural Cheddar Cheese. O. R. IRVINE. Canad. Dairy and Ice Cream Jour., *26*, 6: 27. June, 1947.

The wrapping of prints of cheese in transparent plastic film is a new development. Cheese lends itself remarkably well to wrapping in transparent films. In order to be effective, the package should protect the product's flavor, prevent mold growth, and have a fairly long shelf life. Among other desirable features in the package are low cost and ability to withstand abuse at storage temperatures. The package also should carry colorful, clear, and readily seen branding. The method of packaging in films is called "pressure packing". The cheeses, usually in 20-lb. squares, are cured until the desired state of maturity is reached, after which the blocks are cut out into small prints and made ready for retail sale. H.P.

402. Packaging Cheese Curd. L. H. JONES. (Assigned to Wingfoot Corp.) U. S. Patent 2,424,693, July 29, 1947 (3 claims). U. S. Patent Office Off. Gaz., *600*, 5: 746. 1947.

After removing the press cloth from cheddar-type cheese, the curd is placed in a bag made of thin, limp, moisture-proof and oil-resistant material and placed in a rack designed in such a manner that the cheese may be cured in the container under pressure in an air-free condition. R.W.

CHEMISTRY

403. Pennsylvania Method for Butterfat. J. R. EASTMAN AND G. R. MAYBEE. Canad. Dairy and Ice Cream Jour., *26*, 5: 64. May, 1947.

With raw milk samples testing 4.1–4.4% butterfat by the standard Babcock test, the addition of n-butyl alcohol to the test bottle increased the test to 0.13, 0.20, and 0.26% by the addition of 1.5, 2, and 3 ml. of n-butyl alcohol, respectively. Two milliliters of ammonium hydroxide as specified in the Pennsylvania modification of the Babcock test had no effect on the test. Results confirm that the decrease in reading due to glymol addition helps to offset the increase due to the addition of n-butyl alcohol in the Pennsylvania modification of the Babcock test. The combined effect of glymol and 3 ml. of n-butyl alcohol will cause the results to be about 0.20% higher than those by the Babcock test on raw milk samples of about 4% fat content. H.P.

404. Relationship of Acetone Bodies to "Cowy" Flavor in Milk. D. V. JOSEPHSON AND D. G. KEENEY, Dept. of Dairy Technology, Ohio State University. Milk Dealer, *36*, 10: 40–42. July, 1947.

Experiments are reported showing that much of the "cowy" or "barny" flavored milk received at milk plants during the winter months is due pri-

marily to chemical substances, *i.e.*, acetone bodies, which are secreted in the milk of cows suffering from ketosis or acetonemia. The disease is common in well-managed and high-producing herds and seldom is encountered when cows are on pasture. The data reported also suggest that some of the "feed" flavors observed in mixed milks also may be due to low concentrations (less than 25 p.p.m.) of acetone bodies. C.J.B.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

405. Titratable Acidity of Milk Powders. A. H. White. Canad. Dairy and Ice Cream Jour., *26*, 8: 27. Aug., 1947.

Acidity tests were made on a number of samples of different types of dried milks to determine the probable causes of variations obtained in the acidity tests of the same sample by different laboratories. Variations in the acidity test were found between the top and bottom portions of reconstituted roller-dried milks. The variations were 0.025 to 0.07% after being held for 15 min. or less, and 0.03 to 0.105% after being held for 1 hr. or longer. The top and middle portions of the reconstituted roller-dried milks decreased in acidity as the time of holding increased. Similar tests on spray-dried skim and whole milks showed no variations in acidity even after being held for over 1 hr. Thorough agitation and mixing of reconstituted roller-dried powders is essential if agreement in acidity tests of the same sample is to be obtained in the same laboratory or different laboratories. H.P.

406. How to Make Yoghurt. L. A. Burkey, Division of Dairy Research Laboratories, U. S. Dept. of Agr. Milk Dealer, *36*, 10: 43, 44, 114. July, 1947.

Complete information on yoghurt manufacture is reported. Directions for handling the culture, steps in carrying mother cultures, preparation of the bulk starter, and the materials and equipment needed for the preparation of yoghurt are given. C.J.B.

DISEASES

407. A Clue to the Cause of Milk Fever in the Metabolism of the Springing Cow. A. H. Craige, Jr. Amer. Jour. Vet. Res., *8*, 28: 247–256. July, 1947.

A study of the causes and treatment of milk fever was conducted on a group of low producing cows not particularly susceptible to the ailment. Clinical symptoms of milk fever and also death were induced by artificial alkalization produced by oral and intravenous administration of sodium carbonate to non-parturient cows. Oral administration of Chlor-Ethamine

induces rises in blood calcium and phosphorus in parturient cows. In non-parturient cows Chlor-Ethamine has little or no effect.

A tentative explanation for milk fever is based on the sudden demand for active withdrawal of various electrolytes from the blood stream into the colostrum and suppression of efficient calcium mobilization in the body as a result of a developing alkalosis. Further conjecture suggests that acetonemia or ketosis may be a result of compensatory mechanisms, which in some cases completely counteract the physiologic alkalizing process. T.M.L.

408. Sensitivity to Penicillin of Microorganisms Associated with Bovine Mastitis. J. O. HEISHMAN. Amer. Jour. Vet. Res., *8*, 28: 257–260. July, 1947.

Results of the examination for sensitivity to the action of penicillin of 150 cultures of bacteria isolated from the milk of cows having varying degrees of mastitis are reported. The inhibition range for *Streptococcus agalactiae* and *Streptococcus uberis* varied between 0.031 and 0.125 units of penicillin per cc. of medium. The coliform rods were not affected by 2 units of penicillin per cc. of medium. T.M.L.

409. Treatment of Bovine Anthrax with Penicillin. C. W. RIGGS AND A. C. TEW. Amer. Vet. Med. Assoc. Jour., *111*, 844: 44–46. July, 1947.

Treatment of anthrax by serum alone, serum and penicillin, and penicillin alone are compared. The treatment of 93 cases indicates that penicillin is an effective means of combating the infection and that it is as effective given alone as it is when given in combination with serum. T.M.L.

410. Further Observations on the Virus Diarrhea (New Transmissible Disease) of Cattle. P. OLAFSON AND C. G. RICHARD. Cornell Vet., *37*, 2: 104–107. April, 1947.

The relaton of rinderpest to virus diarrhea is discussed. The antirinderpest serum was used on a small number of calves to combat virus diarrhea without any success. The virus disease probably is independent of rinderpest, although some of the symptoms are very similar. T.M.L.

411. Staphylococcic Abortion in Cattle. W. D. POUNDEN AND W. E. KRAUSS. Amer. Vet. Med. Assoc. Jour., *111*, 844: 35–37. July, 1947.

Two cases of abortion in late pregnancy in dairy cows are described. Both cows conceived to artificial insemination and, at the time of abortion, copius uterine pus exudates were present. Gram-positive cocci were observed in large numbers in all smears. T.M.L.

412. Blood Transfusions in Bovine Anaplasmosis. J. C. Lotze. Amer. Jour. Vet. Res., *8*, 28: 284–289. July, 1947.

Five cases of experimentally produced bovine anaplasmosis were treated by blood transfusion. It is concluded that in certain cases, blood transfusions are indicated due to the inability of the host to regenerate red blood cells rapidly enough to overcome quickly the severe anemia caused by the infection. To be effective, blood must be given in large quantities.

T.M.L.

413. Use of Sodium Sulfonamides as Single Injection Specific Treatment in Foot Rot. C. R. Forman, J. E. Burch, C. E. Dee, L. Kelley, J. E. Mouw, M. B. Teigland, and J. H. Yarborough. Amer. Vet. Med. Assoc. Jour., *111*, 846: 208–214. Sept., 1947.

Results from treatment of 1,500 cases of foot rot with sulfonamides are presented. The intravenous injection of sodium sulfapyridine is the method of choice in treatment of *Actinomyces necrophorus* infections in cows. Treatment is simple and specific. T.M.L.

FEEDS AND FEEDING

414. A Chick Growth Factor in Cow Manure. V. Relation to Quantity and Quality of Soybean Oil Meal in the Diet. M. Rubin and H. R. Bird, Bureau of Animal Industry, Agr. Research Center, Beltsville, Md. Jour. Nutr., *34*, 2: 233. Aug. 11, 1947.

A diet of 70% soybean oil meal caused an inhibition of growth and increased mortality in chicks. These effects were not counteracted by the addition of methionine but were counteracted by the addition of cow manure or cow manure extracts. The growth factor of cow manure improved diets containing heated soybean oil meal more than those containing unheated meal. R.K.W.

FOOD VALUE OF DAIRY PRODUCTS

415. Comparative Nutritive Value of Casein and Lactalbumen for Man. A. J. Mueller and W. M. Cox, Jr., Mead Johnson and Co., Evansville, Ind. Jour. Nutr., *34*, 3: 285. Sept. 10, 1947.

Casein and lactalbumen were fed at an intake of 2% of the calories to 4 adults following 12-day depletion periods. Four-day periods of supplementation with one protein were followed by 4 days of depletion and then 4 days of supplementation with the alternate protein. Each protein was fed for two such 4-day periods. Nitrogen balances and biological values indicate that casein and lactalbumen are equally effective in maintaining nitrogen balances in man. R.K.W.

416. The Sparing Action of Protein on the Pantothenic Acid Requirement of the Rat. II. Urinary and Fecal Excretion of Pantothenic Acid. MARJORIE M. NELSON, F. VAN NOUHUYS, AND H. M. EVANS, Institute of Experimental Biology, Univ. of Calif., Berkeley. Jour. Nutr., *34*, 2: 189. Aug. 11, 1947.

It had been shown that high casein diets had a sparing action on the pantothenic acid requirement of the rat. In these studies the protein contents of diets were varied by using diets containing from 24 to 64% casein. The data indicate that the sparing action for pantothenic acid of the high casein diet is real and not due to decreased urinary excretion or increased intestinal synthesis. R.K.W.

417. The Growth-Promoting Action of Cellulose in Purified Diets for Chicks. F. DAVIS AND G. M. BRIGGS, Univ. of Md. Jour. Nutr., *34*, 3: 295. Sept. 10, 1947.

Supplementation of a simple purified diet, containing casein, with cellulose at levels of 5, 10, and 15% increased gain and feed efficiency for young chicks up to 4 weeks of age. Larger amounts of cellulose decreased growth. R.K.W.

ICE CREAM

418. Fruits and Their Importance in Ice Cream. I. S. BRIGHTMAN, Fruit and Syrup Manufacturers Association. Ice Cream Rev., *31*, 1: 69. Aug., 1947.

Because of the eye and taste appeal which they give to frozen desserts, fruits have been an important factor in building ice cream sales to their present level. Today with more than 300 million lb. of fresh and cold packed fruits available, an adequate supply for use in ice cream is assured. Today fruits are available which are being processed to conform with the Food, Drug and Cosmetic Act. This means that the ice cream manufacturer will be supplied with fruits honestly packed, and which are free from insect infestation, mold, bacteria, or other foreign material. W.J.C.

419. Ice Cream Disher. A. GORDON AND S. G. MOZOLEWSKI. U. S. Patent 2,427,095, Sept 9, 1947 (5 claims). U. S. Patent Office Off. Gaz., *602*, 2: 279. 1947.

A scoop type disher is described with a scraper blade, operated by a pivotally mounted actuating device. R.W.

420. Packaged Ice Cream. F. YOARS. Canad. Dairy and Ice Cream Jour., *26*, 8: 30. Aug., 1947.

Quick freezing and quick hardening of ice cream can be accomplished

within a matter of 20–30 min. by use of the wind tunnel. Quality thus can be improved more statisfactorily than by adding more butterfat, which adds cost to the product. Pre-molded portions eliminate bulk ice cream, which, in turn, eliminates dipping and reduces supervision and dispensing difficulties. H.P.

MILK

421. Farm Milk Cooling Requirements. ANONYMOUS. Milk Dealer, *36*, 10: 116–124. July, 1947.

Results of a survey show that there is a trend toward mechanical cooling of milk on the farm. In the complete survey made of 55 cities in 34 states, only 5 cities reported mechanical cooling on the farm as a present positive requirement. Numerous other cities reported, however, that for all practical purposes such a requirement was in effect because it is impractical to meet temperature requirements unless the producer is equipped with mechanical refrigeration. This is shown by the reports from 52 cities which gave specific information on temperature requirements for milk upon delivery at the plant receiving platform. Of these, 10 reported requirements of 50°, 3 of 55°, 15 of 60°, 4 of 65° and 20 of 70° F. C.J.B.

422. Milk Cooler. G. R. DUNCAN. U. S. Patent 2,425,519, August 12, 1947 (10 claims). U. S. Patent Office Off. Gaz., *601*, 2: 266. 1947.

In a cabinet for cooling milk in cans, a cooling liquid is sprayed around the necks of the cans and down the sides to collect in a sump beneath the cans, to be cooled again and recirculated. R.W.

423. Milk Machine. E. B. THOMPSON AND F. A. GESSLER. (Assigned to Globe Milker, Inc.) U. S. Patent 2,427,312, Sept. 9, 1947 (16 claims). U. S. Patent Office Off. Gaz., *602*, 2: 334. 1947.

This apparatus consists of a self contained receptacle for receiving the milk through a connection from a teatcup, a pair of air chambers operating at subatmospheric pressure, an electrical motor for operating the pump, and an air distributing device. R.W.

424. Frozen Milk with Special Reference to Protein Flocculation. F. J. DOAN AND F. G. WARREN. Canad. Dairy and Ice Cream Jour., *26*, 7: 60. July, 1947.

Milk can be stored in the frozen condition either in the fluid or concentrated state for considerable periods of time. High heat treatment at time of pasteurization delays the onset of oxidation and deterioration of flavor of frozen milk. Stability of frozen milk is decreased by removal of water,

by high heat treatment, by high storage temperature, and by long periods of storage. H.P.

425. **Homogenized Milk, Concentrated Milk Products and Other Developments.** C. A. IVERSON. Canad. Dairy and Ice Cream Jour., *26*, 5: 37. May, 1947.

See Abs. 303, Jour. Dairy Sci., *30*, 9: A129. Sept., 1947.

PHYSIOLOGY

426. **Physiologic Reactions to Intravenous Calcium Injections in the Cow.** A. H. CRAIGE, JR. Amer. Jour Vet. Res., *8*, 28: 260–267. July, 1947.

A series of 16 experiments were conducted by intravenous injection of 500 cc. of calcium gluconate in non-parturient cows. A sudden increase in blood calcium and phosphorus results after intravenous injection, followed by a progressive decline until 24 hr. later, when the normal values are approached. T.M.L.

SANITATION AND CLEANSING

427. **Sanitary Standards for Dairy Equipment.** E. H. PARFITT, Chairman, Sanitary Standards Subcommittee, Dairy Industry Committee. Milk Dealer, *36*, 10: 136–142. July, 1947.

A complete description of the purpose, organization, accomplishments, and of the work in progress of the Sanitary Standards Subcommittee is reported. In conclusion, the author states that: "The dairy industry is now well organized through all its branches to do an outstanding job in arriving at sanitary standards for dairy equipment. The final answer as to the success of this undertaking will depend upon: (1) The backing that is given to these standards by the individual milk sanitarian, and his willingness to go along with these standards rather than attempt to modify them according to his individual thinking. (2) The publicity that is given to these standards so that buyers of equipment for which standards have been developed will specify the accepted 3A sanitary standards. (3) The degree of support that is given by the regulatory groups in seeing to it that fabricators whose equipment does not meet the sanitary standards is not designated or sold as meeting the 3A standard." C.J.B.

428. **The Use of a Quaternary Ammonium Compound as a Supplement to Heat in the Rinse in Mechanical Dishwashing.** W. L. MALLMANN AND L. ZARKOWSKI, Division of Bacteriology and Public Health, Mich. Engin. Expt. Sta., Lansing. Jour. Milk and Food Technol., *10*, 4: 206–213. July–Aug., 1947.

Washing and sanitizing dishes in a mechanical dishwasher in which the

temperature of the rinse water is maintained at 170° F. reduces the health hazards to a safe margin. However, lunch counters and soda fountains frequently resort to hand washing, with the temperature of the rinse water too low to be effective in the destruction of pathogenic organisms. Any attempt to supplement hot water rinsing by using sanitizing agents is highly desirable.

The authors conclude that quaternary ammonium compounds in concentrations of 1–6,400 to 10,000 at a temperature of 120° F. will destroy both Gram-positive and Gram-negative bacteria in 5 to 10 seconds in the absence of organic matter. This procedure is effective in sanitizing machine-washed dishes where high-temperature water is not available or is impractical to use.

H.H.W.

429. Inconsistencies in Testing and Reporting the Strength of Alkaline Detergent Solutions. C. W. ENGLAND. C. Y. Stephens Dairy and Poultry Industries, Washington, D. C. Milk Dealer, *36*, 10: 49–56. July, 1947.

The literature on testing and reporting the strength of alkaline detergent solution is reviewed. This review serves to indicate the duplicity of terms or designations and the confusion relative to reporting results of tests. There is wide diversity in the amount of sample used and the method of analysis. A system of reporting the results of the analysis of alkaline detergent solutions is proposed. C.J.B.

430. D.D.T. and Dairy Sanitation. C. F. GERLACH, Michigan Chemical Corp. Milk Dealer, *36*, 10: 156–160. July, 1947.

Insects may affect dairy products directly by contact and infestation and indirectly by contamination of containers, carrying contaminating organisms, and reducing production and quality. DDT has proved not only an aid to sanitation but a profitable investment. Increased milk production and quality have returned profits many times over and above the cost of the DDT program. Directions are given for controlling flies, lice, ticks, silverfish, roaches, ants, and fleas on dairy farms. Directions also are given for insect control in dairy plants. C.J.B.

431. Elimination of Multiple Inspections and Appraisal for Various Plants and Localities. W. A. WENTWORTH, The Borden Co., New York, N. Y. Milk Indus. Found. Assoc. Bul., *39*, 17: 491–500. June 25, 1947.

Faults in the present milk inspection system and recent experience have suggested the following conclusions: (a) The quality of a milk supply cannot be judged by the ordinances governing its production and processing because

of frequently ineffective enforcement. (b) There are extreme variations in milk quality between areas which have identical ordinances. (c) Re-evaluation and modernization of our milk ordinances should be of immediate concern to the industry and public health officials. The problems of use of milk inspection to raise trade barriers and the burdens of duplicate and conflicting regulations are discussed. The author suggests an effort in the field of city milk supply on the lines of the work of the Dairy Products Improvement Committee for sweet cream. Another industry-wide effort is the work of the Sanitary Standards Committee of the Dairy Industry Committee, which, in conjunction with the International Association of Milk Sanitarians, is developing appropriate standards for milk plant equipment. E.F.G.

MISCELLANEOUS

432. Agricultural Marketing Agreements—A Review. W. C. WELDEN, H. C. Hood and Sons, Inc., Boston, Mass. Milk Indus. Found. Assoc. Bul., *39*, 17 : 483–490. June 25, 1947.

The first license issued Aug. 1, 1933, covered the Chicago Milk Market. Since the inauguration of this practice under the Agricultural Adjustment Act of May, 1933, there have been close to 100 licenses or orders issued by the Secretary.

During the war operation of marketing orders tended to cause more rapid price adjustment under OPA, and these markets were more adequately supplied with milk than other areas. A trend in marketing order techniques is to price milk by formula, which simplifies revision of prices both up and down.

Prospective problems of federal control involve two angles: (a) Can a more balanced approach be developed to point orders toward industry and the public welfare as well as producer welfare? (b) Can the orders be made really effective in promoting steady and adequate milk supplies at reasonable and stable prices? E.F.G.

433. Functions and Responsibilities of Leadership in the Dairy Plant. J. H. ERB. Canad. Dairy and Ice Cream Jour., *26*, 7 : 48. July, 1947.

Competent supervision should be able to control quality so that it is uniformly good day after day. In order to work out a quality problem, the following steps should be taken: (a) Analyze the problem, (b) plan the solution, (c) sell top management on changes, (d) train subordinates, and (e) check back. The maintenance of equipment requires precedence in the time and energy of plant supervision. Plant supervision should play a role in cost control. Ingredients and finished products should be accounted for and handled like money. The proper type of leader will have to be developed

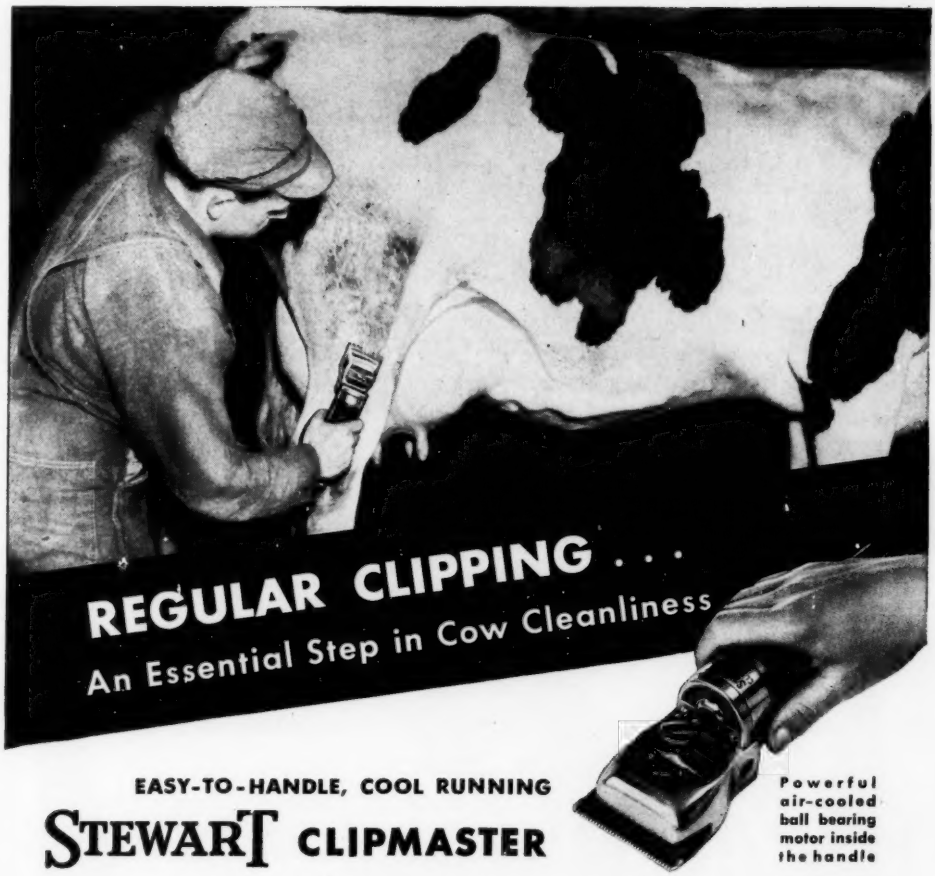
by management by better selection of leaders, providing a training program for developing greater leadership skills, reducing unreasonable load so there is more time to study and work on "problem" areas, and bringing middle supervision closer to management. To qualify for the job of being a leader one must have training, not only past training and experience but training while on the job. H.P.

434. A Few Basic Considerations in Dairy Economics. D. B. DeLoach. Canad. Dairy and Ice Cream Jour., *26*, 7 : 43. July, 1947.

The milk industry of today is the result of the ability of the industry to utilize fully the scientific background of the 19th and early part of the 20th centuries. The milk industry has the unusual advantage of almost universal acceptance of its products as a basic food for children and adults. It has received favorable attention in the health and educational programs of our public school system and has become an item essential to the cookery techniques of every modern homemaker. The components of efficient plant operations will have to center around the following: (a) procurement costs and plant location, (b) product utilization and market prices, (c) plant volume, (d) transportation to market, and (e) packaging. H.P.

435. Problems in the Design and Layouts of Dairy Plants. R. T. Southmayd. Canad. Dairy and Ice Cream Jour., *26*, 8 : 62. Aug., 1947.

The market which a plant will serve and the amount of business it may be expected to do should be used in determining its most efficient and flexible layout. The lot size is very important as it determines the size of the plant, free and easy handling of traffic, good appearance, and friendly relations with neighbors. Three general problems of the plant proper are size and location of the various work spaces, type and location of processing equipment, and type, size, and location of mechanical equipment. All the individual problems must be blended into a co-ordinated unit that will utilize human effort efficiently. H.P.



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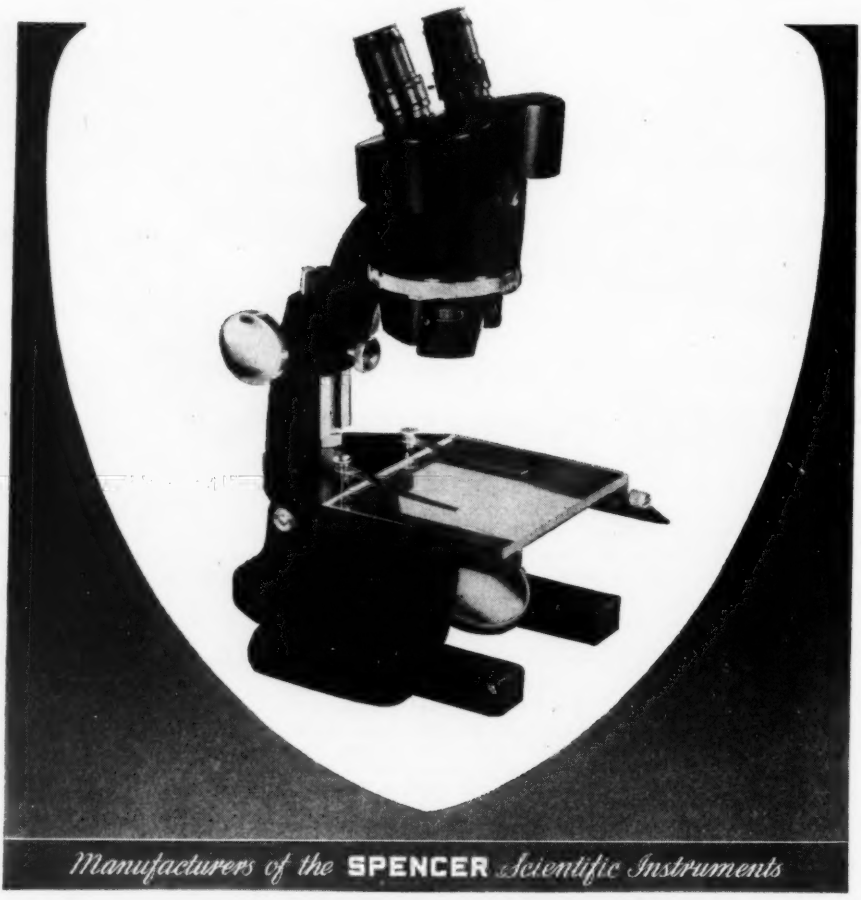

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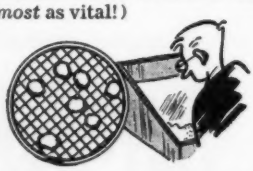

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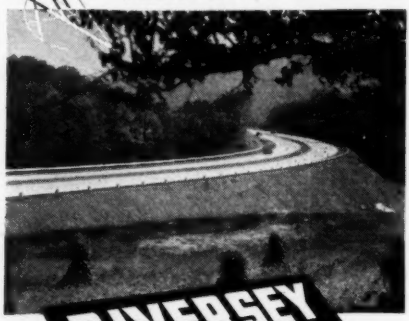

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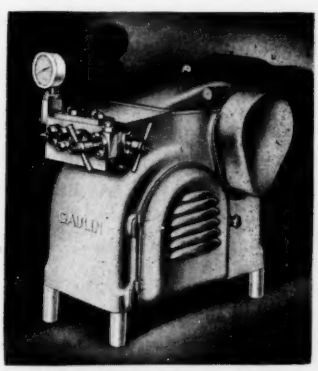

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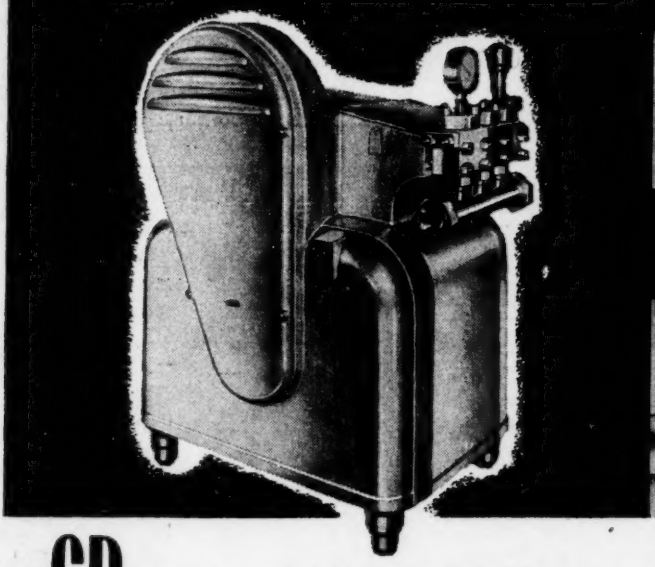

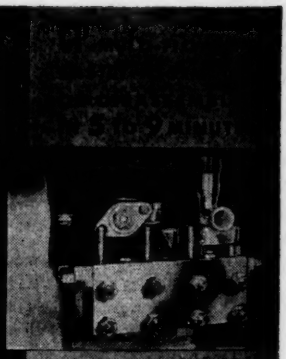

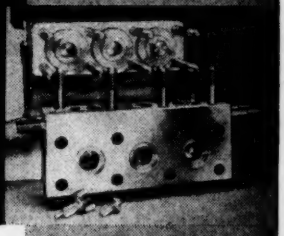

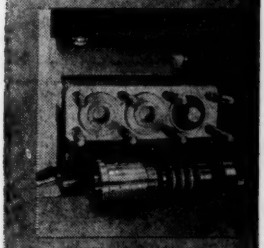

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